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<p>An enzymatic nucleic acid molecule which cleaves RNA associated with development or maintenance of an arthritic condition, induction of graft tolerance or reversal of an immune response. In particular, the ribozyme sequences are directed to an mRNA encoding B7-1, B7-2, B7-3, CD40 and/or stromelysin. Also provided are ribozymes where the uracil in positions 4 and/or 7 are substituted, as well as methods for the synthesis of 2'-alkylnucleotides, 2'-O-alkylthioalkyl, or 2'-alkylthioalkylnucleotides. The application further describes a method for diprotection of RNA with aqueous ethylamine, a method for synthesis of a basic ribonucleoside mimetics, and transcription units comprising an RNA polymerase II promoter, a U6 small nuclear promoter, or an adenovirus VA1 promoter system.</p>																																				

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METHOD AND REAGENT FOR TREATMENT OF ARTHRITIC
CONDITIONS, INDUCTION OF GRAFT TOLERANCE AND
REVERSAL OF IMMUNE RESPONSES

5

Background of the Invention

The following is a discussion of relevant art, none of which is admitted to be prior art to the present invention.

10 In one aspect, this invention relates to methods for inhibition of osteoarthritis, in particular, inhibition of genetic expression which leads to a reduction or elimination of extracellular matrix digestion by matrix metalloproteinases.

15 There are several types of arthritis, with osteoarthritis and rheumatoid arthritis being predominant. Osteoarthritis is a slowly progressive disease characterized by degeneration of articular cartilage with proliferation and remodeling of subchondral bone. It presents with a clinical picture of pain, deformity, and loss of joint motion. Rheumatoid arthritis is a chronic systemic inflammatory disease. Rheumatoid arthritis may be mild and relapsing or severe and progressive, leading to joint deformity and incapacitation.

20 Arthritis is the major contributor to functional impairment among the older population. It is the major cause of disability and accounts for a large proportion of the hospitalizations and health care expenditures of the elderly. Arthritis is estimated to be the principal cause of total incapacitation for about one million persons aged 55 and older and is thought to be an important contributing cause for about one million more.

25 Estimating the incidence of osteoarthritis is difficult for several reasons. First, osteoarthritis is diagnosed objectively on the basis of reading radiographs, but many people with radiologic evidence of disease have no obvious symptoms. Second, the estimates of prevalence are based upon clinical evaluations because radiographic data is not available for all afflicted joints. In the NHANESI survey of 1989, data were based upon a thorough musculoskeletal evaluation during which any abnormalities of the spine, knee,

30

hips, and peripheral joints were noted as well as other specific diagnoses. Based on these observations, 12% of the US population between 25 and 74 years of age have osteoarthritis.

5 It is generally agreed that rheumatoid arthritis has a world-wide distribution and affects all racial and ethnic groups. The exact prevalence in the US is unknown but has been estimated to range between 0.5% and 1.5%. Rheumatoid arthritis occurs at all age levels and generally increases in prevalence with advancing age. It is 2-3 times more prevalent in women than in men and peak incidence occurs between 40-60 years of age. In addition to
10 immunological factors, environmental, occupational and psychosocial factors have been studied for potential etiologic roles in the disease.

The extracellular matrix of multicellular organisms plays an important role in the formation and maintenance of tissues. The meshwork of the extracellular matrix is deposited by resident cells and provides a framework for
15 cell adhesion and migration, as well as a permeability barrier in cell-cell communication. Connective tissue turnover during normal growth and development or under pathological conditions is thought to be mediated by a family of neutral metalloproteinases, which are zinc-containing enzymes that require calcium for full activity. The regulation of metalloproteinase
20 expression is cell-type specific and may vary among species.

The best characterized of the matrix metalloproteinases, interstitial collagenase (MMP-1), is specific for collagen types I, II, and III. MMP-1 cleaves all three chains of the triple helix at a single point initiating sequential breakdown of the interstitial collagens. Interstitial collagenase activity has
25 been observed in rheumatoid synovial cells as well as in the synovial fluid of patients with inflammatory arthritis. Gelatinases (MMP-2) represent a subgroup of the metalloproteinases consisting of two distinct gene products; a 70 kDa gelatinase expressed by most connective tissue cells, and a 92 kDa gelatinase expressed by inflammatory phagocytes and tumor cells. The larger
30 enzyme is expressed by macrophages, SV-40 transformed fibroblasts, and neutrophils. The smaller enzyme is secreted by H-ras transformed bronchial epithelial cells and tumor cells, as well as normal human skin fibroblasts. These enzymes degrade gelatin (denatured collagen) as well as native

collagen type XI. Stromelysin (MMP-3) has a wide spectrum of action on molecules composing the extracellular matrix. It digests proteoglycans, fibronectin, laminin, type IV and IX collagens and gelatin, and can remove the N-terminal propeptide region from procollagen, thus activating the collagenase. It has been found in human cartilage extracts, rheumatoid synovial cells, and in the synovium and chondrocytes of joints in rats with collagen-induced arthritis.

Both osteoarthritis and rheumatoid arthritis are treated mainly with compounds that inhibit cytokine or growth-factor induced synthesis of the matrix metalloproteinases which are involved in the extracellular matrix destruction observed in these diseases. Current clinical treatments rely upon dexamethasone and retinoid compounds, which are potent suppressors of a variety of metalloproteinases. The global effects of dexamethasone and retinoid treatment upon gene expression in treated cells make the development of alternative therapies desirable, especially for long term treatments. Recently, it was shown that gamma-interferon suppressed lipopolysaccharide induced collagenase and stromelysin production in cultured macrophages. Also, tissue growth factor- β (TGF- β) has been shown to block epidermal growth factor (EGF) induction of stromelysin synthesis in vitro. Experimental protocols involving gene therapy approaches include the controlled expression of the metalloproteinase inhibitors TIMP-1 and TIMP-2. Of the latter three approaches, only γ -interferon treatment is currently feasible in a clinical application.

Sullivan and Draper, International PCT Publication No. WO 94/02595 and Draper *et al.*, International PCT Publication No. WO 95/13380 disclose the use of ribozymes to treat arthritis.

In a second aspect, the invention relates to methods for the induction of graft tolerance, treatment of autoimmune diseases, inflammatory disorders and allergies in particular, by inhibition of B7-1, B7-2, B7-3 and CD40.

An adaptive immune response requires activation, clonal expansion, and differentiation of a class of cells termed T lymphocytes (T cells). T cell activation is a multi-step process requiring several signalling events between

the T cell and an antigen presenting cell. The ensuing discussion details signals that are exchanged between T cells and antigen presenting B cells. Similar pathways are thought to occur between T cells and other antigen presenting cells such as monocytes or follicular dendritic cells.

5 T cell activation is initiated when the T-cell receptor (TCR) binds to a specific antigen that is associated with the MHC proteins on the surface of an antigen presenting cell. This primary stimulus activates the T cell and induces expression of CD40 ligand (CD40L) on the surface of the T cell. CD40L then
10 interacts with its cognate receptor, CD40, which is constitutively expressed on the surface of B cells; CD40 transduces the signal leading to B cell activation. B cell activations result in the expression of B7-1, B7-2 and/or B7-3, which in turn interacts with constitutively expressed CD28 on the surface of T cells. The interaction generates a secondary co-stimulatory signal that is required to fully activate the T cell. Complete T cell activation via the T cell receptor and CD28
15 leads to cytokine secretion, clonal expansion, and differentiation. If the T cell receptor is engaged, absence of this secondary co-stimulus mediated by CD28, then the T cell is inactivated, either by clonal anergy (non-responsiveness or reduced reactivity of the immune system to specific antigen(s)) or clonal deletion (Jenkins et al., 1987 *Proc. Natl. Acad. Sci. USA*
20 84, 5409). Thus, engagement of the TCR without a concomitant costimulatory signal results in a state of tolerance toward the specific antigen recognized by the T cell. This co-stimulatory signal can be mediated by the binding of B7-1 or B7-2 or B7-3, present on activated antigen-presenting cells, to CD28, a receptor that is constitutively expressed on the surface of the T cell
25 (Marshall et al., 1993 *J Clin Immun* 13, 165-174; Linsley, et al., 1991 *J Exp Med* 173, 721; Koulouva et al., 1991 *J Exp Med* 173, 759; Harding et al., 1992 *Nature* 356, 607).

 Several homologs of B7 (now known as B7-1; Cohen, 1993 *Science* 262, 844) are expressed in activated B cells (Freeman et al., 1993 *Science*
30 262, 907; Lenschow et al., 1993 *Proc Natl Acad Sci USA* 90, 11054; Azuma et al., 1993 *Nature* 366, 76; Hathcock et al., 1993 *Science* 262, 905; Freeman et al., 1993 *Science* 262, 909). B7-1 and B7-3 are only expressed on the surface of a subset of B cells after 48 hours of contact with T cells. In contrast, B7-2 mRNA is constitutively expressed by unstimulated B cells and increases 4-fold

within 4 hours of activation (Freeman et al., 1993 *Science* 262, 909; Boussiotis et al., 1993 *Proc Natl Acad Sci USA* 90, 11059). Since T cells commit to either the anergy or the activation pathway within 12-24 hours of the initial TCR signal, it is thought that B7-2 is the molecule responsible for the primary
5 costimulatory signal. B7-1 and B7-3 may provide a subsequent signal necessary for clonal expansion. Antibodies to B7-2 completely block T cell proliferation in a mixed lymphocyte reaction (Azuma et al., 1993 *supra*), supporting the central role of B7-2 in T cell activation. These experiments indicate that inhibition of B7-2 expression (for example with a ribozyme) would
10 likely induce anergy. Similarly, inhibition of CD40 expression by a ribozyme would prevent B7-2 upregulation and could induce tolerance to specific antigens.

B7 (B7-1) is a 60 KD modified trans-membrane glycoprotein usually present on the surface of antigen presenting cells (APC). B7 has two ligands--
15 CD28 and CTLA4. Interaction of B7-1 with CD28 and/or CTLA4 causes activation of T cell responses (Janeway and Bottomly, 1994 *Cell* 76, 275).

B7-2 is a 70 KD (34 KD unmodified) trans-membrane glycoprotein found on the surface of APCs. B7-2 encodes a 323 amino-acid protein which is 26 % identical to human B7-1 protein. Like B7-1, CD28 and CTLA4 are
20 selectively bound by B7-2. B7-2, unlike B7-1, is expressed on the surface of unstimulated B cells (Freeman et al., 1993 *supra*).

CD40 is a 45-50 KD surface glycoprotein found on the surface of late pre-B cells in bone marrow, mature B cells, bone marrow-derived dendritic cells and follicular dendritic cells (Clark and Ledbetter, 1994 *Nature* 367, 425).

25 Successful organ transplantation currently requires suppression of the recipient's immune system in order to prevent graft rejection and maintain good graft function. The available therapies, including cyclosporin A, FK506 and various monoclonal antibodies, all have serious side effects (Caine, 1992 *Transplantation Proceedings* 24, 1260; Fuleihan et al., 1994 *J. Clin. Invest.* 93, 1315; Van Gool et al., 1994 *Blood* 83, 176) . In addition, existing therapies
30 result in general immune suppression, leaving the patient susceptible to a variety of opportunistic infections. The ability to induce a state of long-term,

antigen-specific tolerance to the donor tissue would revolutionize the field of organ and tissue transplantation. Since organ graft rejection is mediated by T cell effector function, the goal is to block specifically the activation of the subset of T cells that recognize donor antigens. A limitation in the field of transplantation is the supply of donor organs (Nowak 1994 *Science* 266, 1148). The ability to induce donor-specific tolerance would substantially increase the chances of successful allografts, xenografts, thereby greatly increasing the donor pool.

Such transplantation includes grafting of tissues and/or organ ie., implantation or transplantation of tissue and/or organs, from the body of an individual to a different place within the same or different individual. Transplantation also involve grafting of tissues and/or organs from one area of the body to another. Transplantation of tissues and/or organs between genetically dissimilar animals of the same species is termed as allogeneic transplantation. Transplantation of animal organs into humans is termed xenotransplants (for a review see Nowak, 1994 *Science* 266, 1148).

One therapy currently being developed that has similar potential to induce antigen-specific tolerance is treatment with a CTLA4-Ig fusion protein. "CTLA4" is a homologue of CD28 that binds B7-1 and B7-2 with high affinity. The engineered, soluble fusion protein, CTLA4-Ig, binds B7-1, thereby blocking its interaction with CD28. The results of CTLA4-Ig treatment in animal studies are mixed. CTLA4-Ig treatment significantly enhanced survival rates and ameliorated the symptoms of graft-versus host disease in a murine bone marrow transplant model (Blazer et al., 1994 *Blood* 83, 3815). CTLA4-Ig induced long-term (>110 days) donor-specific tolerance in pancreatic islet xenografts (Lenschow et al., 1992 *Science* 257, 789). Conversely, in another study CTLA4-Ig treatment delayed but did not ultimately prevent cardiac allograft rejection (Turka, et al., 1992 *Proc Natl Acad Sci U S A* 89, 11102). Mice immunized with sheep erythrocytes in the presence of CTLA4-Ig failed to mount a primary immune response (Linsley, et al., 1992 *Science* 257, 792). A secondary immunization did elicit some response, however, indicating incomplete tolerance. Interestingly, identical results were obtained when CTLA4-Ig was administered 2 days after primary immunization, leading the authors to conclude that CTLA4-Ig blocked amplification rather than initiation

of the immune response. Since CTLA4-Ig has been shown to dissociate more rapidly from B7-2 compared with B7-1, this may explain the failure to induce long term tolerance in this model (Linsley et al., 1994 *Immunity* 1, 793).

5 CTLA4:Ig has recently been shown to ameliorate symptoms of spontaneous autoimmune disease in lupus-prone mice (Finck et al., 1994 *Science* 265, 1225).

Linsley et al., WO 92/00092 describe B7 antigen as a ligand for CD28 receptor on T cells. The application states that—

10 "The B7 antigen, or its fragments or derivatives are reacted with CD28 positive T cells to regulate T cell interactions with other cells..... B7 antigen or CD28 receptor may be used to inhibit interaction of cells associated with these molecules, thereby regulating T cell responses."

De Boer and Conroy, WO 94/01547 describe the use of anti-B7 and anti-CD40 antibodies to treat allograft transplant rejection, graft versus host disease and rheumatoid arthritis. The application states that—

15 "...anti-B7 and anti-CD40 antibodies...can be used to prevent or treat an antibody-mediated or immune system disease in a patient."

Since signalling via CD40 precedes induction of B-7, blocking the CD40-CD40L interaction would also have the potential to produce tolerance. According to one report, simultaneous treatment of mice with antibodies to
20 CD40L and sheep red blood cells produced antigen-specific tolerance for up to 3 weeks following cessation of treatment (Foy et al., 1993 *J Exp Med* 178, 1567). Anti-CD40L also produces antigen specific tolerance in a pancreatic islet transplant model (R. Noelle, personal communication). Targeted inhibition of CD40 expression in B cells in addition to B7 would therefore
25 afford double protection against activation of T cells.

Therapeutic agents used to prevent rejection of a transplanted organ are all cytotoxic compounds or antibodies designed to suppress the cell-mediated immune system. The side effects of these agents are those of immunosuppression and infections. The primary approved agents are
30 azathioprine, corticosteroids, cyclosporine; the antibodies are antilymphocyte or antithymocyte globulins. All of these are given to individuals who have been as closely matched as possible to their donors by both major and minor

histocompatibility typing. Since the principal problem in transplantation is an antigenic mismatch and the resulting need for cytotoxic therapy, any therapeutic improvement which decreases the local immune response without general immunosuppression should capture the transplant market.

- 5 Cyclosporine: At the end of the 1970's and early 1980's the introduction of cyclosporine revolutionized the transplantation field. It is a potent immunosuppressant which can inhibit immunocompetent lymphocytes specifically and reversibly. Its primary mechanism of action appears to be inhibition of the production and release of interleukin-2 by T helper cells. In
10 addition it also interferes with the release of interleukin-1 by macrophages, as well as proliferation of B lymphocytes. It was approved by the FDA in 1983 and by 1989 was almost universally given to transplant recipients. At first it was believed that the toxicity and side effects from cyclosporine were minimal and it was hailed as a "wonder drug." Numerous side effects have been
15 progressively cited, including the appearance of lymphomas, especially in the gastrointestinal tract; acute and chronic nephrotoxicity; hypertension; hepatotoxicity; hirsutism; anemia; neurotoxicity; endocrine and neurological complications; and gastrointestinal distress. It is now widely acknowledged that the non-specific side effects of the drug demand caution and close
20 monitoring of its use. One-year survival rates for cadaver kidney transplants treated with cyclosporine is 80%, much better than the 50-60% rates without the drug. The one-year survival is almost 90% for transplants with related donors and the use of cyclosporine.

- 25 Azathioprine: In addition to cyclosporine, azathioprine is used for transplant patients. Azathioprine is one of the mercaptopurine class of drugs and inhibits nucleic acid synthesis. Patients are maintained indefinitely on daily doses of 1mg/kg or less, with a dosage adjusted in accordance with the white cell count. The drug may cause depression of bone marrow elements and may cause jaundice.

- 30 Corticosteroids: Prednisone, used in almost all transplant recipients, is usually given in association with azathioprine and cyclosporine. The dosage must be regulated carefully so as to prevent complications such as infection, development of cushingoid features, and hypertension. Usually the initial

maintenance prednisone dosage is 0.5 mg/kg/d. This dosage is usually further decreased in the outpatient clinic until maintenance levels of about 10 mg/d for adults are obtained. The exact site of action of corticosteroids on the immune response is not known.

- 5 Antithymoblast or antilymphocyte globulin (ALG) and antithymocyte globulin (ATG): These are important adjunctive immunosuppressants. They are effective, particularly in induction of immunosuppressive therapy and in the treatment of corticosteroid-resistant rejection. Both ALG and ATG can be made by immunizing horses, rabbits, or sheep; the main source is horses.
- 10 Lymphocytes from human peripheral blood, spleen, lymph nodes, or thymus serve as the immunogen.

- Tacrolimus: On April 13, 1994 the Food and Drug Administration approved another drug to help prevent the rejection of organ transplants. The drug, tacrolimus, was approved only for use in liver transplant patients. An
- 15 alternative to cyclosporine, the macrolide immunosuppressant tacrolimus is a powerful and selective anti-T-lymphocyte agent that was discovered in 1984. Tacrolimus, isolated from the fungus *Streptomyces tsukubaensis*, possesses immunodepressant properties similar to but more potent than cyclosporine. It inhibits both cell-mediated and humoral immune responses. Like
- 20 cyclosporine, tacrolimus demonstrates considerable interindividual variation in its pharmacokinetic profile. Most clinical studies with tacrolimus have neither been published in their entirety nor subjected to extensive peer review; there is also a paucity of published randomized investigations of tacrolimus vs. cyclosporine, particularly in renal transplantation. Despite these drawbacks,
- 25 tacrolimus has shown notable efficacy as a rescue or primary immunosuppressant therapy when combined with corticosteroids. The potential for reductional withdrawal of corticosteroid therapy with tacrolimus appears to be a distinct advantage compared with the cyclosporine. This benefit may be enhanced by reduced incidence of infectious complications,
- 30 hypertension and hypercholesterolemia reported by some investigators. In other respects, the tolerability profile of tacrolimus appears to be broadly similar to that of cyclosporine.

In addition to induction of graft tolerance, T cell anergy can be used to reverse autoimmune diseases. Autoimmune diseases represent a broad category of conditions. A few examples include insulin-dependent diabetes mellitus (IDDM), multiple sclerosis (MS), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), myasthenia gravis (MG), and psoriasis. These seemingly disparate diseases all share the common feature of inappropriate immune response to specific self-antigens. Finck et al. *supra* have reported that CTLA4Ig treatment of mice blocked auto-antibody production in a mice model of SLE. In fact, this effect was observed even when the CTLA4Ig treatment was initiated during the advanced stages of the disease, suggesting that the autoimmune response was a reversible process.

Chappel, WO 94/11011 describes methods to treat autoimmune diseases by inducing tolerance to cells, tissues and organs. The application states that—

"Cells genetically engineered with DNA encoding a plurality of antigens of a cell, tissue, or organ to which tolerance is to be induced. The cells are free of co-stimulatory antigens, such as B7 antigen. Such cells induce T-cell anergy against the proteins encoded by the DNA, and may be administered to a patient in order to prevent the onset of or to treat an autoimmune disease, or to induce tolerance to a tissue or organ prior to transplantation."

Allergic reactions represent an immediate hypersensitivity response to environmental antigens, typically mediated by IgE antibodies. The ability to induce antigen-specific tolerance provides a powerful avenue to alleviate allergies by exposure to the antigen in conjunction with down-regulation of B7-1, B7-2, B7-3 or CD40.

The specific roles of B7-1, B7-2 and B7-3 in T cell activation remains to be determined. Some studies suggest that their functions are essentially redundant (Hathcock et al 1994 *J Exp. Med.* 180, 631), or that the differences observed in the kinetics of expression might simply indicate that B7-2 is important in the initiation of the co-stimulatory signal, while B7-1 plays a role in the amplification of that signal. Other studies point to more specific functions. For example, Kuchroo et al., 1995 *Cell* 80, 707, have reported that blocking B7-1 expression may favor a Th2 response, while blocking B7-2 expression favors a Th1 response. These two helper T cell subpopulations play distinct roles in the immune response and inflammatory disease. Th1 cells are

strongly correlated with auto-immune disease. Allergic responses are typically triggered by Th2 response. Therefore, the decision to target B7-1, B7-2, CD40 or a combination of the above will depend to the particular disease application.

5

Summary of the Invention

Applicant notes that the inhibition of collagenase and stromelysin production in the synovial membrane of joints can be accomplished using ribozymes and antisense molecules. Ribozyme treatment can be a partner to
10 current treatments which primarily target immune cells reacting to pre-existing tissue damage. Early ribozyme or antisense treatment which reduces the collagenase or stromelysin-induced damage can be followed by treatment with the anti-inflammatories or retinoids, if necessary. In this manner, expression of the proteinases can be controlled at both transcriptional and
15 translational levels. Ribozyme or antisense treatment can be given to patients expressing radiological signs of osteoarthritis prior to the expression of clinical symptoms. Ribozyme or antisense treatment can impact the expression of stromelysin without introducing the non-specific effects upon gene expression which accompany treatment with the retinoids and dexamethasone. The
20 ability of stromelysin to activate procollagenase indicates that a ribozyme or antisense molecule which reduces stromelysin expression can also be used in the treatment of both osteoarthritis (which is primarily a stromelysin-associated pathology) and rheumatoid arthritis (which is primarily related to enhanced collagenase activity).

25 While a number of cytokines and growth factors induce metalloproteinase activities during wound healing and tissue injury of a pre-osteoarthritic condition, these molecules are not preferred targets for therapeutic intervention. Primary emphasis is placed upon inhibiting the molecules which are responsible for the disruption of the extracellular matrix,
30 because most people will be presenting radiologic or clinical symptoms prior to treatment. The most versatile of the metalloproteinases (the molecule which can do the most structural damage to the extracellular matrix, if not regulated)

is stromelysin. Additionally, this molecule can activate procollagenase, which in turn causes further damage to the collagen backbone of the extracellular matrix. Under normal conditions, the conversion of prostromelysin to active stromelysin is regulated by the presence of inhibitors called TIMPs (tissue inhibitors of MMP). Because the level of TIMP in synovial cells exceeds the level of prostromelysin and stromelysin activity is generally absent from the synovial fluid associated with non-arthritic tissues, the toxic effects of inhibiting stromelysin activity in non-target cells should be negligible.

Thus, the invention features use of specific ribozyme molecules to treat or prevent arthritis, particularly osteoarthritis, by inhibiting the synthesis of the prostromelysin molecule in synovial cells, or by inhibition of other matrix metalloproteinases discussed above. Cleavage of targeted mRNAs (stromelysin mRNAs, including stromelysin 1, 2, and 3, and collagenase) expressed in macrophages, neutrophils and synovial cells represses the synthesis of the zymogen form of stromelysin, prostromelysin.

Ribozymes are RNA molecules having an enzymatic activity which is able to repeatedly cleave other separate RNA molecules in a nucleotide base sequence specific manner. It is said that such enzymatic RNA molecules can be targeted to virtually any RNA transcript and efficient cleavage has been achieved *in vitro*. Kim et al., 84 Proc. Nat. Acad. of Sci. USA 8788, 1987; Haseloff and Gerlach, 334 Nature 585, 1988; Cech, 260 JAMA 3030, 1988; and Jefferies et al., 17 Nucleic Acid Research 1371, 1989.

Six basic varieties of naturally-occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds in *trans* (and thus can cleave other RNA molecules) under physiological conditions. Table I summarizes some of the characteristics of these ribozymes. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct

synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

By "enzymatic RNA molecule" it is meant an RNA molecule which has
5 complementarity in a substrate binding region to a specified mRNA target, and also has an enzymatic activity which is active to specifically cleave that mRNA. That is, the enzymatic RNA molecule is able to intermolecularly cleave mRNA and thereby inactivate a target mRNA molecule. This complementarity
10 functions to allow sufficient hybridization of the enzymatic RNA molecule to the target RNA to allow the cleavage to occur. One hundred percent complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention. For *in vivo* treatment, complementarity between 30 and 45 bases is preferred; although lower numbers are also useful.

By "complementary" is meant a nucleotide sequence that can form
15 hydrogen bond(s) with other nucleotide sequence by either traditional Watson-Crick or other non-traditional types (for example Hoogsteen type) of base-paired interactions.

The enzymatic nature of a ribozyme is advantageous over other
20 technologies, such as antisense technology (where a nucleic acid molecule simply binds to a nucleic acid target to block its translation) since the concentration of ribozyme necessary to affect a therapeutic treatment is lower than that of an antisense oligonucleotide. This advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to
25 cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can completely eliminate catalytic activity of a ribozyme. Similar mismatches in antisense molecules do not
30 prevent their action (Woolf, T. M., et al., 1992, Proc. Natl. Acad. Sci. USA, 89, 7305-7309). Thus, the specificity of action of a ribozyme is greater than that of an antisense oligonucleotide binding the same RNA site.

In preferred embodiments of this invention, the enzymatic nucleic acid molecule is formed in a hammerhead or hairpin motif, but may also be formed in the motif of a hepatitis delta virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) or *Neurospora* VS RNA. Examples of such hammerhead motifs are described by Rossi *et al.*, 1992, Aids Research and Human Retroviruses 8, 183, of hairpin motifs by Hampel *et al.*, EPA 0360257, Hampel and Tritz, 1989 Biochemistry 28, 4929, and Hampel *et al.*, 1990 Nucleic Acids Res. 18, 299, and an example of the hepatitis delta virus motif is described by Perrotta and Been, 1992 Biochemistry 31, 16; of the RNaseP motif by Guerrier-Takada *et al.*, 1983 Cell 35, 849, *Neurospora* VS RNA ribozyme motif is described by Collins (Saville and Collins, 1990 Cell 61, 685-696; Saville and Collins, 1991 Proc. Natl. Acad. Sci. USA 88, 8826-8830; Collins and Olive, 1993 Biochemistry 32, 2795-2799) and of the Group I intron by Cech *et al.*, U.S. Patent 4,987,071. These specific motifs are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule.

The invention provides a method for producing a class of enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of a desired target. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target stromelysin encoding mRNAs such that specific treatment of a disease or condition can be provided with either one or several enzymatic nucleic acids. Such enzymatic nucleic acid molecules can be delivered exogenously to specific cells as required. Alternatively, the ribozymes can be expressed from DNA or RNA vectors that are delivered to specific cells.

Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small enzymatic nucleic acid motifs (*e.g.*, of the hammerhead or the hairpin structure) are used for exogenous delivery. The simple structure of these molecules increases the ability of the enzymatic nucleic acid to invade targeted regions of the mRNA structure. However,

these catalytic RNA molecules can also be expressed within cells from eukaryotic promoters (e.g., Scanlon et al., 1991, Proc. Natl. Acad. Sci. USA, 88, 10591-5; Kashani-Sabet et al., 1992 Antisense Res. Dev., 2, 3-15; Dropulic et al., 1992 J. Virol., 66, 1432-41; Weerasinghe et al., 1991 J. Virol., 65, 5531-4; Ojwang et al., 1992 Proc. Natl. Acad. Sci. USA, 89, 10802-6; Chen et al., 1992 Nucleic Acids Res., 20, 4581-9; Sarver et al., 1990 Science 247, 1222-1225; Thompson et al., 1995 Nucleic Acids Res., 23, 2259). Those skilled in the art realize that any ribozyme can be expressed in eukaryotic cells from the appropriate DNA vector. The activity of such ribozymes can be augmented by their release from the primary transcript by a second ribozyme (Draper et al., PCT WO 93/23569, and Sullivan et al., PCT WO 94/02595; Ohkawa et al., 1992 Nucleic Acids Symp. Ser., 27, 15-6; Taira et al., 1991, Nucleic Acids Res., 19, 5125-30; Ventura et al., 1993 Nucleic Acids Res., 21, 3249-55; Chowrira et al., 1994 J. Biol. Chem., 269, 25856) .

15 Ribozyme therapy, due to its exquisite specificity, is particularly well-suited to target mRNA encoding factors that contribute to disease pathology. Thus, ribozymes that cleave stromelysin mRNAs may represent novel therapeutics for the treatment of asthma.

20 Thus, in a first aspect, the invention features ribozymes that inhibit stromelysin production. These chemically or enzymatically synthesized RNA molecules contain substrate binding domains that bind to accessible regions of their target mRNAs. The RNA molecules also contain domains that catalyze the cleavage of RNA. The RNA molecules are preferably ribozymes of the hammerhead or hairpin motif. Upon binding, the ribozymes cleave the target
25 stromelysin encoding mRNAs, preventing translation and stromelysin protein accumulation. In the absence of the expression of the target gene, a therapeutic effect may be observed.

30 By "inhibit" is meant that the activity or level of stromelysin encoding mRNAs and protein is reduced below that observed in the absence of the ribozyme, and preferably is below that level observed in the presence of an inactive RNA molecule able to bind to the same site on the mRNA, but unable to cleave that RNA.

Such ribozymes are useful for the prevention of the diseases and conditions discussed above, and any other diseases or conditions that are related to the level of stromelysin activity in a cell or tissue. By "related" is meant that the inhibition of stromelysin mRNAs and thus reduction in the level of stromelysin activity will relieve to some extent the symptoms of the disease or condition.

Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The RNA or RNA complexes can be locally administered to relevant tissues ex vivo, or in vivo through injection, aerosol inhalation, infusion pump or stent, with or without their incorporation in biopolymers. In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables AII, AIII, AIV, AVI, AVIII and AIX. Examples of such ribozymes are shown in Tables AV, AVII, AVIII and AIX. Examples of such ribozymes consist essentially of sequences defined in these Tables.

By "consists essentially of" is meant that the active ribozyme contains an enzymatic center equivalent to those in the examples, and binding arms able to bind mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage.

In a related aspect the invention features ribozymes that cleave target molecules and inhibit stromelysin activity are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Ribozyme expressing viral vectors could be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. Preferably, the recombinant vectors capable of expressing the ribozymes are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of ribozymes. Such vectors might be repeatedly administered as necessary. Once expressed, the ribozymes cleave the target mRNA. Delivery of ribozyme expressing vectors could be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that would allow for introduction into the desired target cell.

By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

5 This class of chemicals exhibits a high degree of specificity for cleavage of the intended target mRNA. Consequently, the ribozyme agent will only affect cells expressing that particular gene, and will not be toxic to normal tissues.

10 The invention can be used to treat or prevent (prophylactically) osteoarthritis or other pathological conditions which are mediated by metalloproteinase activation. The preferred administration protocol is *in vivo* administration to reduce the level of stromelysin activity.

15 Thus, the invention features an enzymatic RNA molecule (or ribozyme) which cleaves mRNA associated with development or maintenance of an arthritic condition, e.g., mRNA encoding stromelysin, and in particular, those mRNA targets disclosed in the accompanying tables, which include both hammerhead and hairpin target sites. In each case the site is flanked by regions to which appropriate substrate binding arms can be synthesized and an appropriate hammerhead or hairpin motif can be added to provide enzymatic activity, by methods described herein and known in the art. For example, referring to Figure 1, arms I and III are modified to be specific
20 substrate-binding arms, and arm II remains essentially as shown.

25 Ribozymes that cleave stromelysin mRNAs represent a novel therapeutic approach to arthritic disorders like osteoarthritis. The invention features use of ribozymes to treat osteoarthritis, e.g., by inhibiting the synthesis of prostromelysin molecule in synovial cells or by the inhibition of matrix metalloproteinases. Applicant indicates that ribozymes are able to inhibit the secretion of stromelysin and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described that other ribozymes that cleave stromelysin encoding mRNAs may be readily designed and are within the
30 invention.

In other related aspects, the invention features a mammalian cell which includes an enzymatic RNA molecule as described above. Preferably, the

mammalian cell is a human cell; and the invention features an expression vector which includes nucleic acid encoding an enzymatic RNA molecule described above, located in the vector, e.g., in a manner which allows expression of that enzymatic RNA molecule within a mammalian cell; or a
5 method for treatment of a disease or condition by administering to a patient an enzymatic RNA molecule as described above.

The invention provides a class of chemical cleaving agents which exhibit a high degree of specificity for the mRNA causative of an arthritic condition. Such enzymatic RNA molecules can be delivered exogenously or
10 endogenously to infected cells. In the preferred hammerhead motif the small size (less than 40 nucleotides, preferably between 32 and 36 nucleotides in length) of the molecule allows the cost of treatment to be reduced.

The enzymatic RNA molecules of this invention can be used to treat arthritic or prearthritic conditions. Such treatment can also be extended to
15 other related genes in nonhuman primates. Affected animals can be treated at the time of arthritic risk detection, or in a prophylactic manner. This timing of treatment will reduce the chance of further arthritic damage.

In another aspect, the invention features novel nucleic acid-based techniques [e.g., enzymatic nucleic acid molecules (ribozymes), antisense
20 nucleic acids, 2-5A antisense chimeras, triplex DNA, antisense nucleic acids containing RNA cleaving chemical groups (Cook et al., U.S. Patent 5,359,051)] and methods for their use to induce graft tolerance, to treat autoimmune diseases such as lupus, rheumatoid arthritis, multiple sclerosis and to treatment of allergies.

25 In a preferred embodiment, the invention features use of one or more of the nucleic acid-based techniques to induce graft tolerance by inhibiting the synthesis of B7-1, B7-2, B7-3 and CD40 proteins.

Those in the art will recognize the other potential targets, for e.g., ICAM-1, VCAM-1, β 1 integrin (VLA4) are also suitable for treatment with the nucleic
30 acid-based techniques described in the present invention.

By "inhibit" is meant that the activity of B7-1, B7-2, B7-3 and/or CD40 or level of mRNAs encoded by B7-1, B7-2, B7-3 and/or CD40 is reduced below that observed in the absence of the nucleic acid. In one embodiment, inhibition with ribozymes preferably is below that level observed in the presence of an enzymatically inactive RNA molecule able to bind to the same site on the mRNA, but unable to cleave that RNA.

By "equivalent" RNA to B7-1, B7-2, B7-3 and/or CD40 is meant to include those naturally occurring RNA molecules associated with graft rejection in various animals, including human, mice, rats, rabbits, primates and pigs.

By "antisense nucleic acid" is meant a non-enzymatic nucleic acid molecule that binds to another RNA (target RNA) by means of RNA-RNA or RNA-DNA or RNA-PNA (protein nucleic acid; Egholm et al., 1993 *Nature* 365, 566) interactions and alters the activity of the target RNA (for a review see Stein and Cheng, 1993 *Science* 261, 1004).

By "2-5A antisense chimera" is meant, an antisense oligonucleotide containing a 5' phosphorylated 2'-5'-linked adenylate residues. These chimeras bind to target RNA in a sequence-specific manner and activate a cellular 2-5A-dependent ribonuclease which in turn cleaves the target RNA (Torrence et al., 1993 *Proc. Natl. Acad. Sci. USA* 90, 1300).

By "triplex DNA" is meant an oligonucleotide that can bind to a double-stranded DNA in a sequence-specific manner to form a triple-strand helix. Triple-helix formation has been shown to inhibit transcription of the targeted gene (Duval-Valentin et al., 1992 *Proc. Natl. Acad. Sci. USA* 89, 504).

By "gene" is meant a nucleic acid that encodes an RNA.

Ribozymes that cleave the specified sites in B7-1, B7-2, B7-3 and/or CD40 mRNAs represent a novel therapeutic approach to induce graft tolerance and treat autoimmune diseases, allergies and other inflammatory conditions. Applicant indicates that ribozymes are able to inhibit the activity of B7-1, B7-2, B7-3 and/or CD40 and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described that other ribozymes that cleave these

sites in B7-1, B7-2, B7-3 and/or CD40 mRNAs may be readily designed and are within the invention.

In a preferred embodiment the invention provides a method for producing a class of enzymatic cleaving agents which exhibit a high degree of specificity
5 for the RNA of a desired target. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target mRNAs encoding B7-1, B7-2, B7-3 and/or CD40 proteins such that specific treatment of a disease or condition can be provided with either one or several enzymatic nucleic acids. Such enzymatic nucleic acid molecules can be delivered
10 exogenously to specific cells as required. Alternatively, the ribozymes can be expressed from DNA/RNA vectors that are delivered to specific cells.

Such ribozymes are useful for the prevention of the diseases and conditions discussed above, and any other diseases or conditions that are related to the levels of B7-1, B7-2, B7-3 and/or CD40 activity in a cell or tissue.
15 By "related" is meant that the inhibition of B7-1, B7-2, B7-3 and/or CD40 mRNAs and thus reduction in the level respective protein activity will relieve to some extent the symptoms of the disease or condition.

Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The nucleic
20 acid or nucleic acid complexes can be locally administered to relevant tissues ex vivo, or in vivo through injection, infusion pump or stent, with or without their incorporation in biopolymers. In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables BII, BIV, BVI, BVIII, BX, BXII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX. Examples of
25 such ribozymes are shown in Tables BIII, BV, BVI, BVII, BIX, BXI, BXIII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX. Examples of such ribozymes consist essentially of sequences defined in these Tables.

In another aspect of the invention, ribozymes that cleave target molecules and inhibit B7-1, B7-2, B7-3 and/or CD40 activity are expressed
30 from transcription units inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Ribozyme expressing viral vectors could be constructed based on, but not limited to, adeno-

associated virus, retrovirus, adenovirus, or alphavirus. Preferably, the recombinant vectors capable of expressing the ribozymes are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of ribozymes. Such vectors might be repeatedly administered as necessary. Once expressed, the ribozymes cleave the target mRNA. Delivery of ribozyme expressing vectors could be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that would allow for introduction into the desired target cell.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

15

Description of the Preferred Embodiments

The drawings will first briefly be described.

Drawings

Figure 1 is a diagrammatic representation of the hammerhead ribozyme domain known in the art. Stem II can be ≥ 2 base-pairs long.

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Figure 2a is a diagrammatic representation of the hammerhead ribozyme domain known in the art; Figure 2b is a diagrammatic representation of the hammerhead ribozyme as divided by Uhlenbeck (1987, *Nature*, 327, 596-600) into a substrate and enzyme portion; Figure 2c is a similar diagram showing the hammerhead divided by Haseloff and Gerlach (1988, *Nature*, 334, 585-591) into two portions; and Figure 2d is a similar diagram showing the hammerhead divided by Jeffries and Symons (1989, *Nucl. Acids. Res.*, 17, 1371-1371) into two portions.

25

Figure 3 is a diagrammatic representation of the general structure of a hairpin ribozyme. Helix 2 (H2) is provided with a least 4 base pairs (*i.e.*, n is 1, 2, 3 or 4) and helix 5 can be optionally provided of length 2 or more bases

30

(preferably 3 - 20 bases, *i.e.*, *m* is from 1 - 20 or more). Helix 2 and helix 5 may be covalently linked by one or more bases (*i.e.*, *r* is ≥ 1 base). Helix 1, 4 or 5 may also be extended by 2 or more base pairs (*e.g.*, 4 - 20 base pairs) to stabilize the ribozyme structure, and preferably is a protein binding site. In each instance, each N and N' independently is any normal or modified base and each dash represents a potential base-pairing interaction. These nucleotides may be modified at the sugar, base or phosphate. Complete base-pairing is not required in the helices, but is preferred. Helix 1 and 4 can be of any size (*i.e.*, *o* and *p* is each independently from 0 to any number, *e.g.*, 20) as long as some base-pairing is maintained. Essential bases are shown as specific bases in the structure, but those in the art will recognize that one or more may be modified chemically (abasic, base, sugar and/or phosphate modifications) or replaced with another base without significant effect. Helix 4 can be formed from two separate molecules, *i.e.*, without a connecting loop. The connecting loop when present may be a ribonucleotide with or without modifications to its base, sugar or phosphate. "q" is ≥ 2 bases. The connecting loop can also be replaced with a non-nucleotide linker molecule. H, refers to bases A, U or C. Y refers to pyrimidine bases. " - " refers to a chemical bond.

Figure 4 is a representation of the general structure of the hepatitis delta virus ribozyme domain known in the art.

Figure 5 is a representation of the general structure of the self-cleaving VS RNA ribozyme domain.

Figure 6 is a schematic representation of an RNaseH accessibility assay. Specifically, the left side of Figure 6 is a diagram of complementary DNA oligonucleotides bound to accessible sites on the target RNA. Complementary DNA oligonucleotides are represented by broad lines labeled A, B, and C. Target RNA is represented by the thin, twisted line. The right side of Figure 6 is a schematic of a gel separation of uncut target RNA from a cleaved target RNA. Detection of target RNA is by autoradiography of body-labeled, T7 transcript. The bands common to each lane represent uncleaved target RNA; the bands unique to each lane represent the cleaved products.

Figure 7 shows *in vitro* cleavage of stromelysin mRNA by HH ribozymes.

Figure 8 shows inhibition of stromelysin expression by 21HH ribozyme in HS-27 fibroblast cell line.

Figure 9 shows inhibition of stromelysin expression by 463HH ribozyme
5 in HS-27 fibroblast cell line.

Figure 10 shows inhibition of stromelysin expression by 1049HH ribozyme in HS-27 fibroblast cell line.

Figure 11 shows inhibition of stromelysin expression by 1366HH ribozyme in HS-27 fibroblast cell line.

10 Figure 12 shows inhibition of stromelysin expression by 1410HH ribozyme in HS-27 fibroblast cell line.

Figure 13 shows inhibition of stromelysin expression by 1489HH ribozyme in HS-27 fibroblast cell line.

15 Figure 14 shows 1049HH ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

Figure 15 shows 1049HH ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

Figure 16 shows 1049HH ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

20 Figure 17 shows the effect of phosphorothioate substitutions on the catalytic activity of 1049 2'-C-allyl HH ribozyme. A) diagrammatic representation of 1049 hammerhead ribozyme•substrate complex. 1049 U4-C-allyl P=S ribozyme represents a hammerhead containing ribose residues at five positions. The remaining 31 nucleotide positions contain 2'-hydroxyl
25 group substitutions, wherein 30 nucleotides contain 2'-O-methyl substitutions and one nucleotide (U₄) contains 2'-C-allyl substitution. Additionally, five nucleotides within the ribozyme, at the 5' and 3' termini, contain

phosphorothioate substitutions. B) shows the ability of ribozyme described in Fig. 17A to decrease the level of stromelysin RNA in rabbit knee.

Figure 18 is a diagrammatic representation of chemically modified ribozymes targeted against stromelysin RNA. 1049 2'-amino P=S Ribozyme represents a hammerhead containing ribose residues at five positions. The remaining 31 nucleotide positions contain 2'-hydroxyl group substitutions, wherein 29 nucleotides contain 2'-O-methyl substitutions and two nucleotides (U₄ and U₇) contain 2'-amino substitution. Additionally, the 3' end of this ribozyme contains a 3'-3' linked inverted T and four nucleotides at the 5' termini contain phosphorothioate substitutions. Arrow-head indicates the site of RNA cleavage (site 1049). 1363 2'-Amino P=S, Human and Rabbit 1366 2'-Amino P=S ribozymes are identical to the 1049 2'-amino P=S ribozyme except that they are targeted to sites 1363 and 1366 within stromelysin RNAs.

Figure 19 shows 1049 2'-amino P=S ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

Figure 20 shows 1363 2'-amino P=S ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

Figure 21 shows 1366 2'-amino P=S ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

Figures 22a-d are diagrammatic representations of non-limiting examples of base modifications for adenine, guanine, cytosine and uracil, respectively.

Figure 23 is a diagrammatic representation of a position numbered hammerhead ribozyme (according to Hertel *et al.*, *Nucleic Acids Res.* 1992, 20:3252) showing specific substitutions in the catalytic core and substrate binding arms. Compounds 4, 9, 13, 17, 22 and 23 are described in Fig. 24.

Figure 24 is a diagrammatic representation of various nucleotides that can be substituted in the catalytic core of a hammerhead ribozyme.

Figure 25 is a diagrammatic representation of the synthesis of a ribothymidine phosphoramidite.

Figure 26 is a diagrammatic representation of the synthesis of a 5-methylcytidine phosphoramidite.

Figure 27 is a diagrammatic representation of the synthesis of 5-bromouridine phosphoramidite.

5 Figure 28 is a diagrammatic representation of the synthesis of 6-azauridine phosphoramidite.

Figure 29 is a diagrammatic representation of the synthesis of 2,6-diaminopurine phosphoramidite.

10 Figure 30 is a diagrammatic representation of the synthesis of a 6-methyluridine phosphoramidite.

Figure 31 is a representation of a hammerhead ribozyme targeted to site A (HH-A). Site of 6-methyl U substitution is indicated.

15 Figure 32 shows RNA cleavage reaction catalyzed by HH-A ribozyme containing 6-methyl U-substitution (6-methyl-U4). U4, represents a HH-A ribozyme containing no 6-methyl-U substitution.

Figure 33 is a representation of a hammerhead ribozyme targeted to site B (HH-B). Sites of 6-methyl U substitution are indicated.

20 Figure 34 shows RNA cleavage reaction catalyzed by HH-B ribozyme containing 6-methyl U-substitutions at U4 and U7 positions (6-methyl-U4). U4, represents a HH-B ribozyme containing no 6-methyl-U substitution.

Figure 35 is a representation of a hammerhead ribozyme targeted to site C (HH-C). Sites of 6-methyl U substitution are indicated.

25 Figure 36 shows RNA cleavage reaction catalyzed by HH-C ribozyme containing 6-methyl U-substitutions at U4 and U7 positions (6-methyl-U4). U4, represents a HH-C ribozyme containing no 6-methyl-U substitution.

Figure 37 shows 6-methyl-U-substituted HH-A ribozyme-mediated inhibition of rat smooth muscle cell proliferation.

Figure 38 shows 6-methyl-U-substituted HH-C ribozyme-mediated inhibition of stromelysin protein production in human synovial fibroblast cells.

Figure 39 is a diagrammatic representation of the synthesis of pyridin-2-one nucleoside and pyridin-4-one nucleoside phosphoramidite.

5 Figure 40 is a diagrammatic representation of the synthesis of 2-*O*-*t*-Butyldimethylsilyl-5-*O*-dimethoxytrityl-3-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-deoxy-1-phenyl- β -D-ribofuranose phosphoramidite.

10 Figure 41 is a diagrammatic representation of the synthesis of pseudouridine, 2,4,6-trimethoxy benzene nucleoside and 3-methyluridine phosphoramidite.

Figure 42 is a diagrammatic representation of the synthesis of dihydrouridine phosphoramidite.

15 Figure 43 A) is diagrammatic representation of a hammerhead ribozyme targeted to site . . . B) shows RNA cleavage reaction catalyzed by hammerhead ribozyme with modified base substitutions at either position 4 or position 7.

20 Figure 44 shows further kinetic characterization of RNA cleavage reactions catalyzed by HH-B ribozyme (A); HH-B with pyridin-4-one substitution at position 7 (B); and HH-B with phenyl substitution at position 7 (C).

Figure 45 is a diagrammatic representation of the synthesis of 2-*O*-*t*-Butyldimethylsilyl-5-*O*-Dimethoxytrityl-3-*O*-(2-Cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-Deoxy-1-Naphthyl- β -D-Ribofuranose.

25 Figure 46 is a diagrammatic representation of the synthesis of Synthesis of 2-*O*-*t*-Butyldimethylsilyl-5-*O*-Dimethoxytrityl-3-*O*-(2-Cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-Deoxy-1-(*p*-Aminophenyl)- β -D-Ribofuranose.

Figure 47 is a diagrammatic representation of a position numbered hammerhead ribozyme (according to Hertel *et al. Nucleic Acids Res.* 1992, 20, 3252) showing specific substitutions.

5 Figure 48 shows the structures of various 2'-alkyl modified nucleotides which exemplify those of this invention. R groups are alkyl groups, Z is a protecting group.

Figure 49 is a diagrammatic representation of the synthesis of 2'-C-allyl uridine and cytidine.

10 Figure 50 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene uridine.

Figure 51 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene cytidine.

Figure 52 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene adenosine.

15 Figure 53 is a diagrammatic representation of the synthesis of 2'-C-carboxymethylidene uridine, 2'-C-methoxycarboxymethylidene uridine and derivatized amidites thereof. X is CH₃ or alkyl as discussed above, or another substituent.

20 Figure 54 is a diagrammatic representation of the synthesis of 2'-C-allyl uridine and cytidine phosphoramidites.

25 Figure 55 is a diagrammatic representation of the synthesis of 2'-O-alkylthioalkyl nucleosides or non-nucleosides and their phosphoramidites. R is an alkyl as defined above. B is any naturally occurring or modified base bearing any N-protecting group suitable for standard oligonucleotide synthesis (Usman *et al.*, *supra*; Scaringe *et al.*, *supra*), and/or H (non-nucleotide), as described by the publications discussed above. CE is cyanoethyl, DMT is a standard blocking group. Other abbreviations are standard in the art.

Figure 56 is a diagrammatic representation of a hammerhead ribozyme, targeted to site B (HH-B), containing 2'-O-methylthiomethyl substitutions.

Figure 57 shows RNA cleavage activity catalyzed by 2'-O-methylthiomethyl substituted ribozymes. A plot of percent cleaved as a function of time is shown. The reactions were carried out at 37°C in the presence of 40 nM ribozyme, 1 nM substrate and 10 mM MgCl₂. Control HH-B ribozyme contained the following modifications; 29 positions were modified with 2'-O-methyl, U4 and U7 positions were modified with 2'-amino groups, 5 positions contained 2'-OH groups. These modifications of the control ribozyme have previously been shown not to significantly effect the activity of the ribozyme (Usman et al., 1994 *Nucleic Acids Symposium Series* 31, 163).

Figure 58 is a diagrammatic representation of the synthesis of an abasic deoxyribose or ribose non-nucleotide mimetic phosphoramidite.

Figure 59 is a diagrammatic representation of a hammerhead ribozyme targeted to site B (HH-B). Arrow indicates the cleavage site.

Figure 60 is a diagrammatic representation of HH-B ribozyme containing abasic substitutions (HH-Ba) at various positions. Ribozymes were synthesized as described in the application. "X" shows the positions of abasic substitutions. The abasic substitutions were either made individually or in certain combinations.

Figure 61 shows the *in vitro* RNA cleavage activity of HH-B and HH-Ba ribozymes. All RNA, refers to HHA ribozyme containing no abasic substitution. U4 Abasic, refers to HH-Ba ribozyme with a single abasic (ribose) substitution at position 4. U7 Abasic, refers to HH-Ba ribozyme with a single abasic (ribose) substitution at position 7.

Figure 62 shows *in vitro* RNA cleavage activity of HH-B and HH-Ba ribozymes. Abasic Stem II Loop, refers to HH-Ba ribozyme with four abasic (ribose) substitutions within the loop in stem II.

Figure 63 shows *in vitro* RNA cleavage activity of HH-B and HH-Ba ribozymes. 3'-Inverted Deoxyribose, refers to HH-Ba ribozyme with an inverted deoxyribose (abasic) substitution at its 3' terminus.

Figure 64 is a diagrammatic representation of a hammerhead ribozyme targeted to site A (HH-A). Target A is involved in the proliferation of mammalian smooth muscle cells. Arrow indicates the site of cleavage. Inactive version of HH-A contains 2 base-substitutions (G5U and A15.1U) that renders the ribozyme catalytically inactive.

Figure 65 is a diagrammatic representation of HH-A ribozyme with abasic substitution (HH-Aa) at position 4. X, shows the position of abasic substitution.

Figure 66 shows ribozyme-mediated inhibition of rat aortic smooth muscle cell (RASMC) proliferation. Both HH-A and HH-Aa ribozymes can inhibit the proliferation of RASMC in culture. Catalytically inactive HH-A ribozyme shows inhibition which is significantly lower than active HH-A and HH-Aa ribozymes.

Figure 67 is a schematic representation of a two pot deprotection protocol with ethylamine (EA).

Figure 68 shows a strategy used in synthesizing a hammerhead ribozyme from two halves. X and Y represent reactive moieties that can undergo a chemical reaction to form a covalent bond (represented by the solid curved line).

Figure 69 shows various non-limiting examples of reactive moieties that can be placed in the nascent loop region to form a covalent bond to provide a full-length ribozyme. CH₂ can be any linking chain as described above including groups such as methylenes, ether, ethylene glycol, thioethers, double bonds, aromatic groups and others; each n independently is an integer from 0 to 10 inclusive and may be the same or different; each R independently is a proton or an alkyl, alkenyl and other functional groups or conjugates such as peptides, steroids, hormones, lipids, nucleic acid sequences and others that provides nuclease resistance, improved cell association, improved cellular uptake or interacellular localization.

Figure 70 shows non-limiting examples of covalent bonds that can be formed to provide the full length ribozyme. The morpholino group arises from reductive reaction of a dialdehyde, which arises from oxidative cleavage of a ribose at the 3'-end of one half ribozyme with an amine at the 5'-end of the half ribozyme. The amide bond is produced when an acid at the 3'-end of one half ribozyme is coupled to an amine at the 5'-end of the other half ribozyme.

Figure 71 shows non-limiting examples of three ribozymes that were synthesized from coupling reactions of two halves. All three were targeted to the site A of *c-myb* RNA (HH-A). HH-A1 was formed from the reaction of two thiols to provide the disulfide linked ribozyme. HH-A2 and HH-A3 were formed using the morpholino reaction. HH-A2 contains a five atom spacer linking the terminal amine to the 5'-end of the half ribozyme. HH-A3 contains a six carbon spacer linking the terminal amine to the 5'-end of the half ribozyme.

Figure 72 shows comparative cleavage activity of half ribozymes, containing five and six base pair stem II regions, that are not covalently linked vs a full length ribozyme. Assays were carried out under ribozyme excess conditions.

Figure 73 shows comparative cleavage activity of half ribozymes, containing seven and eight base pair stem II regions, that are not covalently linked vs a full length ribozyme. Assays were carried out under ribozyme excess conditions.

Figure 74 shows comparative cleavage assay of HH-A1, HH-A2 and HH-A3 (see Figure 72) formed from crosslinking reactions vs a full length ribozyme control. Assays were carried out under ribozyme excess conditions.

Figure 75. Schematic representation of RNA polymerase III promoter structure. Arrow indicates the transcription start site and the direction of coding region. A, B and C, refer to consensus A, B and C box promoter sequences. I, refers to intermediate cis-acting promoter sequence. PSE, refers to proximal sequence element. DSE, refers to distal sequence element. ATF, refers to activating transcription factor binding element. ?, refers to cis-

acting sequence element that has not been fully characterized. EBER, Epstein-Barr-virus-encoded-RNA. TATA is a box well known in the art.

Figure 76 is a general formula for pol III RNA of this invention.

5 Figure 77 is a diagrammatic representation of a U6-S35 Chimera. The S35 motif and the site of insertion of a desired RNA are indicated. This chimeric RNA transcript is under the control of a U6 small nuclear RNA (snRNA) promoter.

10 Figure 78 is a diagrammatic representation of a U6-S35-ribozyme chimera. The chimera contains a hammerhead ribozyme targeted to site I (HHI).

Figure 79 is a diagrammatic representation of a U6-S35-ribozyme chimera. The chimera contains a hammerhead ribozyme targeted to site II (HHII).

15 Figure 80 shows RNA cleavage reaction catalyzed by a synthetic hammerhead ribozyme (HHI) and by an *in vitro* transcript of U6-S35-HHI hammerhead ribozyme.

Figure 81 shows stability of U6-S35-HHII RNA transcript in 293 mammalian cells as measured by actinomycin D assay.

20 Figure 82 is a diagrammatic representation of an adenovirus VA1 RNA. Various domains within the RNA secondary structure are indicated.

25 Figure 83 A shows a secondary structure model of a VA1-S35 chimeric RNA containing the promoter elements A and B box. The site of insertion of a desired RNA and the S35 motif are indicated. The transcription unit also contains a stable stem (S35-like motif) in the central domain of the VA1 RNA which positions the desired RNA away from the main transcript as an independent domain. 83B shows a VA1-chimera which consists of the terminal 75 nt of a VA1 RNA followed by the HHI ribozyme.

Figure 84 shows a comparison of stability of VA1-chimeric RNA vs VA1-S35-chimeric RNA as measured by actinomycin D assay. VA1-chimera

consists of terminal 75 nt of VA1 RNA followed by HHI ribozyme. VA1-S35-chimera structure and sequence is shown in Figure 83.

Ribozymes

5 Ribozymes in one aspect of this invention block to some extent stromelysin expression and can be used to treat disease or diagnose such disease. Ribozymes are delivered to cells in culture and to cells or tissues in animal models of osteoarthritis (Hembry et al., 1993 Am. J. Pathol. 143, 628). Ribozyme cleavage of stromelysin encoding mRNAs in these systems may prevent inflammatory cell function and alleviate disease symptoms.

10 Other ribozymes of this invention block to some extent B7-1, B7-2, B7-3 and/or CD40 production and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture, to cells or tissues in animal models of transplantation, autoimmune diseases and/or allergies and to human cells or tissues *ex vivo* or *in vivo*. Ribozyme cleavage of B7-1, B7-2
15 and/or CD40 encoded mRNAs in these systems may alleviate disease symptoms.

Target sites

Targets for useful ribozymes can be determined as disclosed in Draper et al supra, Sullivan et al., supra, as well as by Draper et al., WO 95/13380 and
20 Stinchcomb et al WO 95/23225. Rather than repeat the guidance provided in those documents here, below are provided specific examples of such methods, not limiting to those in the art. Ribozymes to such targets are designed as described in those applications and synthesized to be tested *in vitro* and *in vivo*, as also described. Such ribozymes can also be optimized
25 and delivered as described therein. While specific examples to mouse, rabbit and other animal RNA are provided, those in the art will recognize that the equivalent human RNA targets described can be used as described below. Thus, the same target may be used, but binding arms suitable for targeting human RNA sequences are present in the ribozyme. Such targets may also
30 be selected as described below.

5 The sequence of human and rabbit stromelysin mRNA were screened for accessible sites using a computer folding algorithm. Potential hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables AII, AIII, AIV, AVI, AVIII and AIX (All sequences are 5' to 3' in the tables.). While rabbit and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, rabbit targeted ribozymes are useful to test efficacy of action of the ribozyme prior to testing in humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

10 Similarly, the sequence of human and mouse B7-1, B7-2, B7-3 and/or CD40 mRNAs were screened for optimal ribozyme target sites using a computer folding algorithm. Hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables BII, BIV, BVI, BVIII, BX, BXII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX (All sequences are 5' to 3' in the
15 tables) The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme. While mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, mouse targeted ribozymes may be useful to test efficacy of action of the ribozyme prior to testing in
20 humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

Hammerhead or hairpin ribozymes are designed that could bind and are individually analyzed by computer folding (Jaeger et al., 1989 Proc. Natl. Acad. Sci. USA, 86, 7706-7710) to assess whether the ribozyme sequences
25 fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. Varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

30 Referring to Figure 6, mRNA is screened for accessible cleavage sites by the method described generally in Draper WO 93/23569. Briefly, DNA oligonucleotides representing potential hammerhead or hairpin ribozyme cleavage sites are synthesized. A polymerase chain reaction is used to

generate a substrate for T7 RNA polymerase transcription from human or rabbit stromelysin cDNA clones. Labeled RNA transcripts are synthesized *in vitro* from the two templates. The oligonucleotides and the labeled transcripts are annealed, RNaseH is added and the mixtures are incubated for the designated times at 37°C. Reactions are stopped and RNA separated on sequencing polyacrylamide gels. The percentage of the substrate cleaved is determined by autoradiographic quantitation using a PhosphorImaging system. From these data, hammerhead ribozyme sites are chosen as the most accessible.

10 Ribozymes of the hammerhead or hairpin motif are designed to anneal to various sites in the mRNA message. The binding arms are complementary to the target site sequences described above. The ribozymes are chemically synthesized. The method of synthesis used follows the procedure for normal RNA synthesis as described in Usman et al., 1987 J. Am. Chem. Soc., 109, 7845-7854 and in Scaringe et al., 1990 Nucleic Acids Res., 18, 5433-5441; Wincott et al., 1995 Nucleic Acids Res., 23, 2677, and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were >98%. Inactive ribozymes were synthesized by substituting a U for G5 and a U for A14 (numbering from Hertel et al., 1992 Nucleic Acids Res., 20, 3252). Hairpin ribozymes are synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 Nucleic Acids Res., 20, 2835-2840). All ribozymes are modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 25 1992 TIBS 17, 34 and Beigelman et al., 1995 J. Biol. Chem. 270, 25702). Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography (HPLC; See Stinchcomb et al., supra) and are resuspended in water.

30 The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables AV, AVII, AVIII and AIX and in Tables BIII, BV, BVI, BVII, BIX, BXI, BXIII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the

binding arms) is altered to affect activity. For example, stem loop II sequence of hammerhead ribozymes listed in Tables AV and AVII (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. Similarly, stem-loop AIV sequence of hairpin ribozymes listed in Tables AVI and AVII (5'-CACGUUGUG-3') can be altered (substitution, deletion and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. The sequences listed in Tables AV, AVII, AVIII and AIX may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

Optimizing Ribozyme Activity

Ribozyme activity can be optimized as described by Stinchcomb *et al.*, *supra*. The details will not be repeated here, but include altering the length of the ribozyme binding arms (stems I and III, see Figure 2c), or chemically synthesizing ribozymes with modifications that prevent their degradation by serum ribonucleases (see *e.g.*, Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 *Nature* 344, 565; Pieken *et al.*, 1991 *Science* 253, 314; Usman and Cedergren, 1992 *Trends in Biochem. Sci.* 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162, as well as Stinchcomb *et al.*, *supra*, Sproat, European Patent Application 92110298.4 and U.S. Patent 5,334,711; Jennings *et al.*, WO 94/13688 and Beigelman *et al.*, *supra* which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules). Modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times and reduce chemical requirements.

Sullivan, *et al.*, *supra*, describes the general methods for delivery of enzymatic RNA molecules. Ribozymes may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. For some

indications, ribozymes may be directly delivered *ex vivo* to cells or tissues with or without the aforementioned vehicles. Alternatively, the RNA/vehicle combination is locally delivered by direct inhalation, by direct injection or by use of a catheter, infusion pump or stent. Other routes of delivery include, but
5 are not limited to, intravascular, intramuscular, subcutaneous or joint injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of ribozyme delivery and administration are provided in Sullivan et al., supra and Draper et al., supra which have been incorporated by reference herein.

10 In another preferred embodiment, the ribozyme is administered to the site of B7-1, B7-2, B7-3 and/or CD40 expression (APC) in an appropriate liposomal vesicle. APCs isolated from donor (for example) are treated with the ribozyme preparation (or other nucleic acid therapeutics) *ex vivo* and the treated cells are infused into recipient. Alternatively, cells, tissues or organs
15 are directly treated with nucleic acids of the present invention prior to transplantation into a recipient.

Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA expression vector. Transcription of the ribozyme sequences are driven from a
20 promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase
25 promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990 Proc. Natl. Acad. Sci. U S A, 87, 6743-7; Gao and Huang 1993 Nucleic Acids Res., 21, 2867-72; Lieber et al., 1993 Methods Enzymol., 217, 47-66; Zhou et al., 1990 Mol. Cell. Biol., 10, 4529-37). Several investigators have
30 demonstrated that ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet et al., 1992 Antisense Res. Dev., 2, 3-15; Ojwang et al., 1992 Proc. Natl. Acad. Sci. U S A, 89, 10802-6; Chen et al., 1992 Nucleic Acids Res., 20, 4581-9; Yu et al., 1993 Proc. Natl. Acad. Sci. U S A, 90, 6340-4; L'Huillier et al., 1992 EMBO J. 11, 4411-8; Lisiewicz et

al., 1993 Proc. Natl. Acad. Sci. U. S. A., 90, 8000-4; Thompson *et al.*, *supra*). The above ribozyme transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated vectors), or viral RNA vectors (such as retroviral or alphavirus vectors).

In a preferred embodiment of the invention, a transcription unit expressing a ribozyme that cleaves stromelysin RNA is inserted into a plasmid DNA vector or an adenovirus DNA virus or adeno-associated virus (AAV) vector. Both viral vectors have been used to transfer genes to the lung and both vectors lead to transient gene expression (Zabner *et al.*, 1993 Cell 75, 207; Carter, 1992 Curr. Opin. Biotech. 3, 533). The adenovirus vector is delivered as recombinant adenoviral particles. The DNA may be delivered alone or complexed with vehicles (as described for RNA above). The recombinant adenovirus or AAV particles are locally administered to the site of treatment, *e.g.*, through incubation or inhalation *in vivo* or by direct application to cells or tissues *ex vivo*.

Specifically useful modifications, optimization and synthetic methods will now be described.

20 Base Modifications

The following discussion of relevant art is dependent on the diagram shown in Figure 1, in which the numbering of various nucleotides in a hammerhead ribozyme is provided.

Odoi *et al.*, *FEBS* 1990, 267:150, state that substitution of guanosine (G) at position 5 of a hammerhead ribozyme for inosine greatly reduces catalytic activity, suggesting "the importance of the 2-amino group of this guanosine for catalytic activity."

Fu and McLaughlin, *Proc. Natl. Acad. Sci. (USA)* 1992, 89:3985, state that deletion of the 2-amino group of the guanosine at position 5 of a hammerhead ribozyme, or deletion of either of the 2'-hydroxyl groups at

position 5 or 8, resulted in ribozymes having a decrease in cleavage efficiency.

5 Fu and McLaughlin, *Biochemistry* 1992, 31:10941, state that substitution of 7-deazaadenosine for adenosine residues in a hammerhead ribozyme can cause reduction in cleavage efficiency. They state that the "results suggest that the N⁷-nitrogen of the adenosine (A) at position 6 in the hammerhead ribozyme/substrate complex is critical for efficient cleavage activity." They go on to indicate that there are five critical functional groups located within the tetrameric sequence GAUG in the hammerhead ribozyme.

10 Slim and Gait, 1992, *BBRC* 183, 605, state that the substitution of guanosine at position 12, in the core of a hammerhead ribozyme, with inosine inactivates the ribozyme.

15 Tuschl *et al.*, 1993 *Biochemistry* 32, 11658, state that substitution of guanosine residues at positions 5, 8 and 12, in the catalytic core of a hammerhead, with inosine, 2-aminopurine, xanthosine, isoguanosine or deoxyguanosine cause significant reduction in the catalytic efficiency of a hammerhead ribozyme.

20 Fu *et al.*, 1993 *Biochemistry* 32, 10629, state that deletion of guanine N⁷, guanine N² or the adenine N⁶-nitrogen within the core of a hammerhead ribozyme causes significant reduction in the catalytic efficiency of a hammerhead ribozyme.

25 Grasby *et al.*, 1993 *Nucleic Acids Res.* 21, 4444, state that substitution of guanosine at positions 5, 8 and 12 positions within the core of a hammerhead ribozyme with O⁶-methylguanosine results in an approximately 75-fold reduction in *k*_{cat}.

Seela *et al.*, 1993 *Helvetica Chimica Acta* 76, 1809, state that substitution of adenine at positions 13, 14 and 15, within the core of a hammerhead ribozyme, with 7-deazaadenosine does not significantly decrease the catalytic efficiency of a hammerhead ribozyme.

Adams *et al.*, 1994 *Tetrahedron Letters* 35, 765, state that substitution of uracil at position 17 within the hammerhead ribozyme-substrate complex with 4-thiouridine results in a reduction in the catalytic efficiency of the ribozyme by 50 percent.

5 Ng *et al.*, 1994 *Biochemistry* 33, 12119, state that substitution of adenine at positions 6, 9 and 13 within the catalytic core of a hammerhead ribozyme with isoguanosine, significantly decreases the catalytic activity of the ribozyme.

10 Jennings *et al.*, U.S. Patent 5,298,612, indicate that nucleotides within a "minizyme" can be modified. They state-

15 "Nucleotides comprise a base, sugar and a monophosphate group. Accordingly, nucleotide derivatives or modifications may be made at the level of the base, sugar or monophosphate groupings..... Bases may be substituted with various groups, such as halogen, hydroxy, amine, alkyl, azido, nitro, phenyl and the like."

20 WO93/23569, WO95/06731, WO95/04818, and WO95/133178 describe various modifications that can be introduced into ribozyme structures.

25 This invention relates to production of enzymatic RNA molecules or ribozymes having enhanced or reduced binding affinity and enhanced enzymatic activity for their target nucleic acid substrate by inclusion of one or more modified nucleotides in the substrate binding portion of a ribozyme such as a hammerhead, hairpin, VS ribozyme or hepatitis delta virus derived
30 ribozyme. Applicant has recognized that only small changes in the extent of base-pairing or hydrogen bonding between the ribozyme and substrate can have significant effect on the enzymatic activity of the ribozyme on that substrate. Thus, applicant has recognized that a subtle alteration in the extent of hydrogen bonding along a substrate binding arm of a ribozyme can be used
35 to improve the ribozyme activity compared to an unaltered ribozyme containing no such altered nucleotide. Thus, for example, a guanosine base may be replaced with an inosine to produce a weaker interaction between a ribozyme and its substrate, or a uracil may be replaced with a bromouracil (BrU) to increase the hydrogen bonding interaction with an adenosine. Other

examples of alterations of the four standard ribonucleotide bases are shown in Figures 22a-d with weaker or stronger hydrogen bonding abilities shown in each figure.

- In addition, applicant has determined that base modification within some catalytic core nucleotides maintains or enhances enzymatic activity compared to an unmodified molecule. Such nucleotides are noted in Figure 23. Specifically, referring to Figure 23, the preferred sequence of a hammerhead ribozyme in a 5' to 3' direction of the catalytic core is CUG ANG A G•C GAA A, wherein N can be any base or may lack a base (abasic); G•C is a base-pair.
- 5 The nature of the base-paired stem II (Figures 1, 2 and 23) and the recognition arms of stems I and III are variable. In this invention, the use of base-modified nucleotides in those regions that maintain or enhance the catalytic activity and/or the nuclease resistance of the hammerhead ribozyme are described. (Bases which can be modified include those shown in capital letters).
- 10
- 15 Examples of base-substitutions useful in this invention are shown in Figure 22, 24-30, 39-43, 45-46. In preferred embodiments cytidine residues are substituted with 5-alkylcytidines (e.g., 5-methylcytidine, Figure 24, R=CH₃, 9), and uridine residues with 5-alkyluridines (e.g., ribothymidine (Figure 24, R=CH₃, 4) or 5-halouridine (e.g., 5-bromouridine, Figure 24, X=Br, 13) or
- 20 6-azapyrimidines (Figure 24, 17) or 6-alkyluridine (Figure 30). Guanosine or adenosine residues may be replaced by diaminopurine residues (Figure 24, 22) in either the core or stems. In those bases where none of the functional groups are important in the complexing of magnesium or other functions of a ribozyme, they are optionally replaced with a purine ribonucleoside (Figure
- 25 24, 23), which significantly reduces the complexity of chemical synthesis of ribozymes, as no base-protecting group is required during chemical incorporation of the purine nucleus. Furthermore, as discussed above, base-modified nucleotides may be used to enhance the specificity or strength of binding of the recognition arms with similar modifications. Base-modified
- 30 nucleotides, in general, may also be used to enhance the nuclease resistance of the catalytic nucleic acids in which they are incorporated. These modifications within the hammerhead ribozyme motif are meant to be non-limiting example. Those skilled in the art will recognize that other ribozyme

motifs with similar modifications can be readily synthesized and are within the scope of this invention.

Substitutions of sugar moieties as described in the art cited above, may also be made to enhance catalytic activity and/or nuclease stability.

5 The invention provides ribozymes having increased enzymatic activity in vitro and in vivo as can be measured by standard kinetic assays. Thus, the kinetic features of the ribozyme are enhanced by selection of appropriate modified bases in the substrate binding arms. Applicant recognizes that while strong binding to a substrate by a ribozyme enhances specificity, it may also
10 prevent separation of the ribozyme from the cleaved substrate. Thus, applicant provides means by which optimization of the base pairing can be achieved. Specifically, the invention features ribozymes with modified bases with enzymatic activity at least 1.5 fold (preferably 2 or 3 fold) or greater than the unmodified corresponding ribozyme. The invention also features a
15 method for optimizing the kinetic activity of a ribozyme by introduction of modified bases into a ribozyme and screening for those with higher enzymatic activity. Such selection may be in vitro or in vivo. By enhanced activity is meant to include activity measured in vivo where the activity is a reflection of both catalytic activity and ribozyme stability. In this invention, the product of
20 these properties is increased or not significantly (less than 10 fold) decreased in vivo compared to an all RNA ribozyme.

By "enzymatic portion" is meant that part of the ribozyme essential for cleavage of an RNA substrate.

25 By "substrate binding arm" is meant that portion of a ribozyme which is complementary to (*i.e.*, able to base-pair with) a portion of its substrate. Generally, such complementarity is 100%, but can be less if desired. For example, as few as 10 bases out of 14 may be base-paired. Such arms are shown generally in Figures 1-3 as discussed below. That is, these arms contain sequences within a ribozyme which are intended to bring ribozyme
30 and target RNA together through complementary base-pairing interactions; *e.g.*, ribozyme sequences within stems I and III of a standard hammerhead ribozyme make up the substrate-binding domain (see Figure 1).

By "unmodified nucleotide base" is meant one of the bases adenine, cytosine, guanosine, uracil joined to the 1' carbon of β -D-ribo-furanose. The sugar also has a phosphate bound to the 5' carbon. These nucleotides are bound by a phosphodiester between the 3' carbon of one nucleotide and the 5' carbon of the next nucleotide to form RNA.

By "modified nucleotide base" is meant any nucleotide base which contains a modification in the chemical structure of an unmodified nucleotide base which has an effect on the ability of that base to hydrogen bond with its normal complementary base, either by increasing the strength of the hydrogen bonding or by decreasing it (e.g., as exemplified above for inosine and bromouracil). Other examples of modified bases include those shown in Figures 22a-d and other modifications well known in the art, including heterocyclic derivatives and the like.

In preferred embodiments the modified ribozyme is a hammerhead, hairpin VS ribozyme or hepatitis delta virus derived ribozyme, and the hammerhead ribozyme includes between 32 and 40 nucleotide bases. The selection of modified bases is most preferably chosen to enhance the enzymatic activity (as observed in standard kinetic assays designed to measure the kinetics of cleavage) of the selected ribozyme, i.e., to enhance the rate or extent of cleavage of a substrate by the ribozyme, compared to a ribozyme having an identical nucleotide base sequence without any modified base.

By "kinetic assays" or "kinetics of cleavage" is meant an experiment in which the rate of cleavage of target RNA is determined. Often a series of assays are performed in which the concentrations of either ribozyme or substrate are varied from one assay to the next in order to determine the influence of that parameter on the rate of cleavage.

By "rate of cleavage" is meant a measure of the amount of target RNA cleaved as a function of time.

Enzymatic nucleic acid having a hammerhead configuration and modified bases which maintain or enhance enzymatic activity are provided. Such nucleic acid is also generally more resistant to nucleases than

unmodified nucleic acid. By "modified bases" in this aspect is meant those shown in Figure 22 A-D, and 24, 30, and 42B or their equivalents; such bases may be used within the catalytic core of the enzyme as well as in the substrate-binding regions. In particular, the invention features modified
5 ribozymes having a base substitution selected from pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyluracil, dihydrouracil, naphthyl, 6-methyl-uracil and aminophenyl. As noted above, substitution in the core may decrease in vitro activity but enhances stability. Thus, in vivo the activity may not be significantly lowered. As exemplified
10 herein such ribozymes are useful in vivo even if active over all is reduced 10 fold. Such ribozymes herein are said to "maintain" the enzymatic activity on all RNA ribozyme.

Small scale synthesis were conducted on a 394 Applied Biosystems, Inc. synthesizer using a modified 2.5 μ mol scale protocol with a 5 min coupling step for
15 alkylsilyl protected nucleotides and 2.5 min coupling step for 2'-O-methylated nucleotides. Table CII outlines the amounts, and the contact times, of the reagents used in the synthesis cycle. A 6.5-fold excess (163 μ L of 0.1 M = 16.3 μ mol) of phosphoramidite and a 24-fold excess of S-ethyl tetrazole (238 μ L of 0.25 M = 59.5 μ mol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle.
20 Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, were 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer: detritylation solution was 2% TCA in methylene chloride (ABI); capping was performed with 16% N-methyl imidazole in THF (ABI) and 10% acetic
25 anhydride/10% 2,6-lutidine in THF (ABI); oxidation solution was 16.9 mM I₂, 49 mM pyridine, 9% water in THF (Millipore). B & J Synthesis Grade acetonitrile was used directly from the reagent bottle. S-Ethyl tetrazole solution (0.25 M in acetonitrile) was made up from the solid obtained from American International Chemical, Inc.

30 Deprotection of the RNA was performed as follows. The polymer-bound oligoribonucleotide, trityl-off, was transferred from the synthesis column to a 4mL glass screw top vial and suspended in a solution of methylamine (MA) at 65 °C for 10 min. After cooling to -20 °C, the supernatant was removed from the polymer support. The support was washed three times with 1.0 mL of

EtOH:MeCN:H₂O/3:1:1, vortexed and the supernatant was then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, were dried to a white powder.

5 The base-deprotected oligoribonucleotide was resuspended in anhydrous TEA•HF/NMP solution (250 µL of a solution of 1.5mL *N*-methylpyrrolidinone, 750 µL TEA and 1.0 mL TEA•3HF to provide a 1.4M HF concentration) and heated to 65°C for 1.5 h. The resulting, fully deprotected, oligomer was quenched with 50 mM TEAB (9 mL) prior to anion exchange desalting.

10 For anion exchange desalting of the deprotected oligomer, the TEAB solution was loaded onto a Qiagen 500[®] anion exchange cartridge (Qiagen Inc.) that was prewashed with 50 mM TEAB (10 mL). After washing the loaded cartridge with 50 mM TEAB (10 mL), the RNA was eluted with 2 M TEAB (10 mL) and dried down to a white powder.

15 Inactive hammerhead ribozymes were synthesized by substituting a U for G₅ and a U for A₁₄ (numbering from (Hertel, K. J., *et al.*, 1992, *Nucleic Acids Res.*, 20, 1232)).

The average stepwise coupling yields were >98% (Wincott *et al.*, 1995 *Nucleic Acids Res.* 23, 2677-2684).

20 Hairpin ribozymes are synthesized either as one part or in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 *Nucleic Acids Res.*, 20, 2835-2840).

Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography (HPLC; See Stinchcomb *et al.*, International PCT Publication No. WO 95/23225, and are resuspended in water.

25 Various modifications to ribozyme structure can be made to enhance the utility of ribozymes. Such modifications will enhance shelf-life, half-life in vitro, stability, and ease of introduction of such ribozymes to the target site, e.g., to enhance penetration of cellular membranes, and confer the ability to recognize and bind to targeted cells.

Examples of such ribozymes are provided in Usman et al., WO 95/13378 and below.

2'-deoxy-2'-nucleotides

Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 Nature 344, 565; Pieken *et al.*, 1991 Science 253, 314; Usman and Cedergren, 1992 Trends in Biochem. Sci. 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162, as well as Stinchcomb *et al.*, *supra*, Sproat, European Patent Application 92110298.4 and U.S. Patent 5,334,711; Jennings *et al.*, WO 94/13688 and Beigelman *et al.*, *supra* which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules. Usman *et al.* also describe various required ribonucleotides in a ribozyme, and methods by which such nucleotides can be defined. De Mesmaeker *et al.* Syn. Lett. 1993, 677-680 (not admitted to be prior art to the present invention) describes the synthesis of certain 2'-C-alkyl uridine and thymidine derivatives. They conclude that "...their use in an antisense approach seems to be very limited."

This invention relates to the use of 2'-deoxy-2'-alkylnucleotides in oligonucleotides, which are particularly useful for enzymatic cleavage of RNA or single-stranded DNA, and also as antisense oligonucleotides. As the term is used in this application, 2'-deoxy-2'-alkylnucleotide-containing enzymatic nucleic acids are catalytic nucleic acid molecules that contain 2'-deoxy-2'-alkylnucleotide components replacing, but not limited to, double stranded stems, single stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript.

Also within the invention are 2'-deoxy-2'-alkylnucleotides which may be present in enzymatic nucleic acid or even in antisense oligonucleotides. Contrary to the findings of De Mesmaeker *et al.* applicant has found that such

nucleotides are useful since they enhance the stability of the antisense or enzymatic molecule, and can be used in locations which do not affect the desired activity of the molecule. That is, while the presence of the 2'-alkyl group may reduce binding affinity of the oligonucleotide containing this modification, if that moiety is not in an essential base pair forming region then the enhanced stability that it provides to the molecule is advantageous. In addition, while the reduced binding may reduce enzymatic activity, the enhanced stability may make the loss of activity of less consequence. Thus, for example, if a 2'-deoxy-2'-alkyl-containing molecule has 10% the activity of the unmodified molecule, but has 10-fold higher stability *in vivo* then it has utility in the present invention. The same analysis is true for antisense oligonucleotides containing such modifications. The invention also relates to novel intermediates useful in the synthesis of such nucleotides and oligonucleotides (examples of which are shown in the Figures 48-54), and to methods for their synthesis.

Thus, the invention features 2'-deoxy-2'-alkylnucleotides, that is a nucleotide base having at the 2'-position on the sugar molecule an alkyl moiety, and in preferred embodiments features those where the nucleotide is not uridine or thymidine. That is, the invention preferably includes all those nucleotides useful for making enzymatic nucleic acids or antisense molecules that are not described by the art discussed above.

Examples of various alkyl groups useful in this invention are shown in Figure 48, where each R group is any alkyl. These examples are not limiting in the invention. Specifically, an "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino, or SH. The term also includes alkenyl groups which are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may

be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂, halogen, N(CH₃)₂, amino, or SH. The term "alkyl" also includes alkynyl groups which have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino or SH.

5 The term "alkyl" does not include alkoxy groups which have an "-O-alkyl" group, where "alkyl" is defined as described above, where the O is adjacent the 2'-position of the sugar molecule.

Such alkyl groups may also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group which has at least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

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In other aspects, also related to those discussed above, the invention features oligonucleotides having one or more 2'-deoxy-2'-alkylnucleotides (preferably not a 2'-alkyl- uridine or thymidine); e.g. enzymatic nucleic acids having a 2'-deoxy-2'-alkylnucleotide; and a method for producing an

enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic molecule with at least one nucleotide having at its 2'-position an alkyl group. In other related aspects, the invention features 2'-deoxy-2'-alkylnucleotide triphosphates.

- 5 These triphosphates can be used in standard protocols to form useful oligonucleotides of this invention.

The 2'-alkyl derivatives of this invention provide enhanced stability to the oligonucleotides containing them. While they may also reduce absolute activity in an *in vitro* assay they will provide enhanced overall activity *in vivo*.

- 10 Below are provided assays to determine which such molecules are useful. Those in the art will recognize that equivalent assays can be readily devised.

- In another aspect, the invention features hammerhead motifs having enzymatic activity having ribonucleotides at locations shown in Figure 47 at 5, 6, 8, 12, and 15.1, and having substituted ribonucleotides at other positions in the core and in the substrate binding arms if desired. (The term "core" refers to positions between bases 3 and 14 in Figure 47, and the binding arms correspond to the bases from the 3'-end to base 15.1, and from the 5'-end to base 2). Applicant has found that use of ribonucleotides at these five locations in the core provide a molecule having sufficient enzymatic activity even when modified nucleotides are present at other sites in the motif. Other such combinations of useful ribonucleotides can be determined as described by Usman *et al. supra*.

2'-O-alkylthioalkyl and 2'-C-alkylthioalkyl containing nucleic acids

- Medina et al., 1988 *Tetrahedron Letters* 29, 3773, describe a method to convert alcohols to methylthiomethyl ethers.

Matteucci et al., 1990 *Tetrahedron Letters*, 31, 2385, report the synthesis of 3'-5'-methylene bond via a methylthiomethyl precursor.

- Veeneman et al., 1990 *Recl. Trav. Chim. Pays-Bas* 109, 449, report the synthesis of 3'-O-methylthiomethyl deoxynucleoside during the synthesis of a dimer containing 3'-5'-methylene bond.

Jones et al., 1993 *J. Org. Chem.* 58, 2983, report the use of 3'-O-methylthiomethyl deoxynucleoside to synthesize a dimer containing a 3'-thioformacetal internucleoside linkages. The paper also describes a method to synthesize phosphoramidites for DNA synthesis.

- 5 Zavgorodny et al., 1991 *Tetrahedron Letters* 32, 7593, describe a method to synthesize a nucleoside containing methylthiomethyl modification.

This invention relates to the incorporation of 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotides or non-nucleotides into nucleic acids, which are particularly useful for enzymatic cleavage of RNA or single-stranded DNA, and
10 also as antisense oligonucleotides.

As the term is used in this application, 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotide or non-nucleotide-containing enzymatic nucleic acids are catalytic nucleic molecules that contain 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotide or non-nucleotides components replacing one or
15 more bases or regions including, but not limited to, those bases in double stranded stems, single stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can
20 also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript.

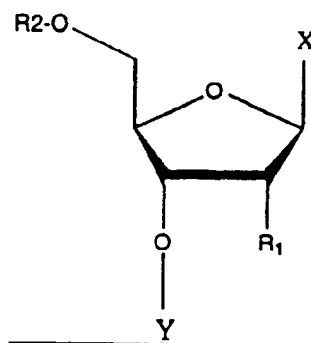
Also within the invention are 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotides or non-nucleotides which may be present in enzymatic nucleic acid or in antisense oligonucleotides or 2-5A antisense chimera. Such
25 nucleotides or non-nucleotides are useful since they enhance the activity of the antisense or enzymatic molecule. The invention also relates to novel intermediates useful in the synthesis of such nucleotides or non-nucleotides and oligonucleotides (examples of which are shown in the Figures), and to methods for their synthesis.

30 Thus, the invention features 2'-O-alkylthioalkyl nucleosides or non-nucleosides, that is a nucleoside or non-nucleosides having at the 2'-position on the sugar molecule a 2'-O-alkylthioalkyl moiety. In a related aspect, the

invention also features 2'-O-alkylthioalkyl nucleotides or non-nucleotides. That is, the invention preferably includes those nucleotides or non-nucleotides having 2' substitutions as noted above useful for making enzymatic nucleic acids or antisense molecules that are not described by the art discussed above.

The term non-nucleotide refers to any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound is abasic in that it does not contain a commonly recognized nucleotide base, such as adenine, guanine, cytosine, uracil or thymine. It may have substitutions for a 2' or 3' H or OH as described in the art. See Eckstein et al. and Usman et al., *supra*.

The term nucleotide refers to the regular nucleotides (A, U, G, T and C) and modified nucleotides such as 6-methyl U, inosine, 5-methyl C and others. Specifically, the term "nucleotide" is used as recognized in the art to include natural bases, and modified bases well known in the art. Such bases are generally located at the 1' position of a sugar moiety. The term "non-nucleotide" as used herein to encompass sugar moieties lacking a base or having other chemical groups in place of a base at the 1' position. Such molecules generally include those having the general formula:



wherein, R1 represents 2'-O-alkylthioalkyl or 2'-C-alkylthioalkyl; X represents a base or H; Y represents a phosphorus-containing group; and R2 represents H, DMT or a phosphorus-containing group (Figure 55).

Phosphorus-containing group is generally a phosphate, thiophosphate, H-phosphonate, methylphosphonate, phosphoramidite or other modified group known in the art.

In another aspect, the invention features 2'-C-alkylthioalkyl nucleosides or non-nucleosides, that is a nucleotide or a non-nucleotide residue having at the 2'-position on the sugar molecule a 2'-C-alkylthioalkyl moiety. In a related aspect, the invention also features 2'-C-alkylthioalkyl nucleotides or non-nucleotides. That is, the invention preferably includes all those 2' modified nucleotides or non-nucleotides useful for making enzymatic nucleic acids or antisense molecules as described above that are not described by the art discussed above.

Specifically, an "alkyl" group is as defined above, except that the term includes 2'-O-alkyl moieties.

In other aspects, also related to those discussed above, the invention features oligonucleotides having one or more 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotides or non-nucleotides; e.g. enzymatic nucleic acids having a 2'-O-methylthiomethyl and/or 2'-C-alkylthioalkyl nucleotides or non-nucleotides; and a method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic molecule with at least one nucleotide or a non-nucleotide moiety having at its 2'-position an 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl group.

In other related aspects, the invention features 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotide triphosphates. These triphosphates can be used in standard protocols to form useful oligonucleotides of this invention.

The 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl derivatives of this invention provide enhanced activity and stability to the oligonucleotides containing them.

In yet another preferred embodiment, the invention features oligonucleotides having one or more 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl abasic (non-nucleotide) moieties. For example, enzymatic

nucleic acids having a 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl abasic moiety; and a method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic molecule with at least one position having at its 2'-
5 position an 2'-O-alkylthioalkyl or 2'-C-alkylthioalkyl group.

In related embodiments, the invention features enzymatic nucleic acids containing one or more 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl substitutions either in the enzymatic portion, substrate binding portion or both, as long as the catalytic activity of the ribozyme is not significantly decreased.

10 In yet another preferred embodiment, the invention features the use of 2'-O-alkylthioalkyl moieties as protecting groups for 2'-hydroxyl positions of ribofuranose during nucleic acid synthesis.

While this invention is applicable to all oligonucleotides, applicant has found that the modified molecules of this invention are particularly useful for
15 enzymatic RNA molecules. Thus, below is provided examples of such molecules. Those in the art will recognize that equivalent procedures can be used to make other molecules without such enzymatic activity. Specifically, Figure 1 shows base numbering of a hammerhead motif in which the numbering of various nucleotides in a hammerhead ribozyme is provided.

20 Referring to Figure 1, the preferred sequence of a hammerhead ribozyme in a 5'- to 3'-direction of the catalytic core is CUGANGAG [base paired with] CGAAA. In this invention, the use of 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl substituted nucleotides or non-nucleotides that maintain or enhance the catalytic activity and or nuclease resistance of the hammerhead
25 ribozyme is described. Substitutions of any nucleotide with any of the modified nucleotides or non-nucleotides discussed above are possible. Usman *et al.*, *supra* and Sproat *et al.*, *supra* as well as other publications indicate those bases that can be substituted in noted ribozyme motifs. Those in the art can thus determine those bases that may be substituted as described
30 herein with beneficial retainment of enzymatic activity and stability.

Non-nucleotides

Usman, et al., WO 93/15187 in discussing modified structures in ribozymes states:

5 It should be understood that the linkages between
the building units of the polymeric chain may be
linkages capable of bridging the units together for
either in vitro or in vivo. For example the linkage
may be a phosphorous containing linkage, e.g.,
10 phosphodiester or phosphothioate, or may be a
nitrogen containing linkage, e.g., amide. It should
further be understood that the chimeric polymer
may contain non-nucleotide spacer molecules
along with its other nucleotide or analogue units.
15 Examples of spacer molecules which may be used
are described in Nielsen et al. Science, 254:1497-
1500 (1991).

Jennings et al., WO 94/13688 while discussing hammerhead ribozymes lacking the usual stem II base-paired region state:

20 One or more ribonucleotides and/or
deoxyribonucleotides of the group (X)_m. [stem II]
may be replaced, for example, with a linker
selected from optionally substituted
polyphosphodiester (such as poly(1-phospho-3-
25 propanol)), optionally substituted alkyl, optionally
substituted polyamide, optionally substituted glycol,
and the like. Optional substituents are well known
in the art, and include alkoxy (such as methoxy,
ethoxy and propoxy), straight or branch chain lower
alkyl such as C₁ - C₅ alkyl), amine, aminoalkyl
30 (such as amino C₁ - C₅ alkyl), halogen (such as F,
Cl and Br) and the like. The nature of optional
substituents is not of importance, as long as the
resultant endonuclease is capable of substrate
cleavage.

35 Additionally, suitable linkers may comprise
polycyclic molecules, such as those containing
phenyl or cyclohexyl rings. The linker (L) may be a
polyether such as polyphosphopropanediol,
polyethyleneglycol, a bifunctional polycyclic
40 molecule such as a bifunctional pentalene, indene,
naphthalene, azulene, heptalene, biphenylene,
asymindacene, sym-indacene, acenaphthylene,
fluorene, phenalene, phenanthrene, anthracene,
fluoranthene, acephenathrylene, aceanthrylene,

5 triphenylene, pyrene, chrysene, naphthacene, thianthrene, isobenzofuran, chromene, xanthene, phenoxathiin, indolizine, isoindole, 3-H-indole, indole, 1-H-indazole, 4-H-quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, 4- α H-carbazole, carbazole, B-carboline, phenanthridine, acridine, perimidine, phenanthroline, phenazine, phenolthiazine, phenoxazine, which polycyclic
10 compound may be substituted or modified, or a combination of the polyethers and the polycyclic molecules.

The polycyclic molecule may be substituted of polysubstituted with C₁-C₅ alkyl, alkenyl, hydroxyalkyl, halogen or haloalkyl group or with O-A or CH₂-O-A wherein A is H or has the formula CONR'R" wherein R' and R" are the same or different and are hydrogen or a substituted or unsubstituted C₁-C₆ alkyl, aryl, cycloalkyl, or heterocyclic group; or A has the formula -M-NR'R" wherein R' and R" are the same or different and are hydrogen, or a C₁-C₅ alkyl, alkenyl, hydroxyalkyl, or haloalkyl group wherein the halo atom is fluorine, chlorine, bromine, or iodine atom; and -M- is an organic moiety having 1 to 10 carbon atoms and is a branched or straight chain alkyl, aryl, or cycloalkyl group.
15 20 25

In one embodiment, the linker is tetraphosphopropanediol or pentaphosphopropanediol. In the case of polycyclic molecules there will be preferably 18 or more atoms bridging the nucleic acids. More preferably their will be from 30 to 50 atoms bridging, see for Example 5. In another embodiment the linker is a bifunctional carbazole or bifunctional carbazole linked to one or more polyphosphoropropanediol.
30 35

Such compounds may also comprise suitable functional groups to allow coupling through reactive groups on nucleotides."
40

This invention concerns the use of non-nucleotide molecules as spacer elements at the base of double-stranded nucleic acid (e.g., RNA or DNA) stems (duplex stems) or more preferably, in the single-stranded regions, catalytic core, loops, or recognition arms of enzymatic nucleic acids. Duplex
45

stems are ubiquitous structural elements in enzymatic RNA molecules. To facilitate the synthesis of such stems, which are usually connected via single-stranded nucleotide chains, a base or base-pair mimetic may be used to reduce the nucleotide requirement in the synthesis of such molecules, and to confer nuclease resistance (since they are non-nucleic acid components). This also applies to both the catalytic core and recognition arms of a ribozyme. In particular abasic nucleotides (i.e., moieties lacking a nucleotide base, but having the sugar and phosphate portions) can be used to provide stability within a core of a ribozyme, e.g., at U4 or N7 of a hammerhead structure shown in Figure 1.

Thus, the invention features an enzymatic nucleic acid molecule having one or more non-nucleotide moieties, and having enzymatic activity to cleave an RNA or DNA molecule.

Examples of such non-nucleotide mimetics are shown in Figure 58 and their incorporation into hammerhead ribozymes is shown in Figure 60. These non-nucleotide linkers may be either polyether, polyamine, polyamide, or polyhydrocarbon compounds. Specific examples include those described by Seela and Kaiser, *Nucleic Acids Res.* 1990, 18:6353 and *Nucleic Acids Res.* 1987, 15:3113; Cload and Schepartz, *J. Am. Chem. Soc.* 1991, 113:6324; Richardson and Schepartz, *J. Am. Chem. Soc.* 1991, 113:5109; Ma et al., *Nucleic Acids Res.* 1993, 21:2585 and *Biochemistry* 1993, 32:1751; Durand et al., *Nucleic Acids Res.* 1990, 18:6353; McCurdy et al., *Nucleosides & Nucleotides* 1991, 10:287; Jäschke et al., *Tetrahedron Lett.* 1993, 34:301; Ono et al., *Biochemistry* 1991, 30:9914; Arnold et al., International Publication No. WO 89/02439 entitled "Non-nucleotide Linking Reagents for Nucleotide Probes"; and Ferentz and Verdine, *J. Am. Chem. Soc.* 1991, 113:4000, all hereby incorporated by reference herein.

In preferred embodiments, the enzymatic nucleic acid includes one or more stretches of RNA, which provide the enzymatic activity of the molecule, linked to the non-nucleotide moiety.

In preferred embodiments, the enzymatic nucleic acid includes one or more stretches of RNA, which provide the enzymatic activity of the molecule,

linked to the non-nucleotide moiety. The necessary ribonucleotide components are known in the art, see, e.g., Usman, supra and Usman et al., Nucl. Acid. Symp. Genes 31:163, 1994.

5 As the term is used in this application, non-nucleotide-containing enzymatic nucleic acid means a nucleic acid molecule that contains at least one non-nucleotide component which replaces a portion of a ribozyme, e.g., but not limited to, a double-stranded stem, a single-stranded "catalytic core" sequence, a single-stranded loop or a single-stranded recognition sequence. These molecules are able to cleave (preferably, repeatedly cleave) separate
10 RNA or DNA molecules in a nucleotide base sequence specific manner. Such molecules can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript. Such molecules also include nucleic acid molecules having a 3' or 5' non-nucleotide, useful as a capping group to prevent exonuclease digestion.

15 Non-nucleotide mimetics useful in this invention are generally described above and in Usman et al. WO 95/06731. Those in the art will recognize that these mimetics can be incorporated into an enzymatic molecule by standard techniques at any desired location. Suitable choices can be made by standard experiments to determine the best location, e.g., by synthesis of the
20 molecule and testing of its enzymatic activity. The optimum molecule will contain the known ribonucleotides needed for enzymatic activity, and will have non-nucleotides which change the structure of the molecule in the least way possible. What is desired is that several nucleotides can be substituted by one non-nucleotide to save synthetic steps in enzymatic molecule synthesis
25 and to provide enhanced stability of the molecule compared to RNA or even DNA.

Synthesis

This invention relates to the synthesis, deprotection, and purification of enzymatic RNA or modified enzymatic RNA molecules in milligram to kilogram
30 quantities with high biological activity. Such syntheses are generally detailed in Stinchcomb et al., WO 95/23225.

This invention relates to the synthesis, deprotection, and purification of enzymatic RNA or modified enzymatic RNA molecules in milligram to kilogram quantities with high biological activity.

Generally, RNA is synthesized and purified by methodologies based on:
5 tetrazole to activate the RNA amidite, NH_4OH to remove the exocyclic amino
protecting groups, tetra-*n*-butylammonium fluoride (TBAF) to remove the 2'-OH
alkylsilyl protecting groups, and gel purification and analysis of the
deprotected RNA. In particular this applies to, but is not limited to, a certain
class of RNA molecules, ribozymes. These may be formed either chemically
10 or using enzymatic methods. Examples of the chemical synthesis,
deprotection, purification and analysis procedures are provided by Usman et
al., 1987 *J. American Chem. Soc.*, 109, 7845, Scaringe et al. *Nucleic Acids*
Res. 1990, 18, 5433-5341, Perreault et al. *Biochemistry* 1991, 30 4020-4025,
and Slim and Gait *Nucleic Acids Res.* 1991, 19, 1183-1188. Odai et al. *FEBS*
15 *Lett.* 1990, 267, 150-152 describes a reverse phase chromatographic
purification of RNA fragments used to form a ribozyme. All the above noted
references are all hereby incorporated by reference herein.

The aforementioned chemical synthesis, deprotection, purification and
analysis procedures are time consuming (10-15 m coupling times) and may
20 also be affected by inefficient activation of the RNA amidites by tetrazole, time
consuming (6-24 h) and incomplete deprotection of the exocyclic amino
protecting groups by NH_4OH , time consuming (6-24 h), incomplete and
difficult to desalt TBAF-catalyzed removal of the alkylsilyl protecting groups,
time consuming and low capacity purification of the RNA by gel
25 electrophoresis, and low resolution analysis of the RNA by gel
electrophoresis.

Imazawa and Eckstein, 1979 *J. Org. Chem.*, 12, 2039, describe the
synthesis of 2'-amino-2'-deoxyribofuranosyl purines. They state that—

30 “To protect the 2'-amino function, we selected the trifluoroacetyl group which can
easily be removed.”

Chemical linkage

Jennings et al., US Patent No. 5,298,612 describe the use of non-nucleotides to assemble a hammerhead ribozyme lacking a stem II portion.

Draper et al., WO 93/23569 (PCT/US93/04020) describes synthesis of
5 ribozymes in two parts in order to aid in the synthetic process (see, e.g., p. 40).

Usman et al., WO 95/06731, describe enzymatic nucleic acid molecules having non-nucleotides within their structure. Such non-nucleotides can be used in place of nucleotides to allow formation of an enzymatic nucleic acid.

This invention relates to improved methods for synthesis of enzymatic
10 nucleic acids and, in particular, hammerhead and hairpin motif ribozymes. This invention is advantageous over iterative chemical synthesis of ribozymes since the yield of the final ribozyme can be significantly increased. Rather than synthesizing, for example, a 37mer hammerhead ribozyme, two partial ribozyme portions, e.g., a 20mer and a 17mer, can be synthesized in
15 significantly higher yield, and the two reacted together to form the desired enzymatic nucleic acid.

Referring to Fig. 68, the strategy involved is shown for a hammerhead ribozyme where each n or n' is independently any desired nucleotide or non-nucleotide, each filled-in circle represents pairing between bases or other
20 entities, and the solid line represents a covalent bond. Within the structure each n and n' may be a ribonucleotide, a 2'-methoxy-substituted nucleotide, or any other type of nucleotide which does not significantly affect the desired enzymatic activity of the final product (see Usman et al., supra). In the particular embodiment shown, which is not limiting in this invention, five
25 ribonucleotides are provided at rG5, rA6, rG8, rG12, and rA15.1. U4 and U7 may be abasic (i.e., lacking the uridine moiety) or may be ribonucleotides, 2'-methoxy substituted nucleotides, or other such nucleotides. a9, a13, and a14 are preferably 2'-methoxy or may have other substituents. The synthesis of this hammerhead ribozyme is performed by synthesizing a 3' and a 5' portion
30 as shown in a lower part of Fig. 68. Each 5' and 3' portion has a chemically reactive group X and Y, respectively. Non-limiting examples of such chemically reactive groups are provided in Fig. 69. These groups undergo

chemical reactions to provide the bonds shown in Fig. 69. Thus, the X and Y can be used, in various combinations, in this invention to form a chemical linkage between two ribozyme portions.

Thus, the invention features a method for synthesis of an enzymatically
5 active nucleic acid (as defined by Draper, supra) by providing a 3' and a 5' portion of that nucleic acid, each having independently chemically reactive groups at the 5' and 3' positions, respectively. The reaction is performed under conditions in which a covalent bond is formed between the 3' and 5' portions by those chemically reactive groups. The bond formed can be, but is
10 not limited to, either a disulfide, morpholino, amide, ether, thioether, amine, a double bond, a sulfonamide, carbonate, hydrazone or ester bond. The bond is not the natural bond formed between a 5' phosphate group and a 3' hydroxyl group which is made during normal synthesis of an oligonucleotide. In other embodiments, more than two portions can be linked together using
15 pairs of X and Y groups which allow proper formation of the ribozyme (see Figure 69).

By "chemically reactive group" is simply meant a group which can react with another group to form the desired bonds. These bonds may be formed under any conditions which will not significantly affect the structure of the
20 resulting enzymatic nucleic acid. Those in the art will recognize that suitable protecting groups can be provided on the ribozyme portions.

In preferred embodiments the nucleic acid has a hammerhead motif and the 3' and 5' portions each have chemically reactive groups in or immediately adjacent to the stem II region (see Fig. 1). The stem II region is evident in Fig.
25 1 between the bases termed a9 and rG12. The C and G within this stem defines the end of the stem II region. Thus, any of the n or n' moieties within the stem II region can be provided with a chemically reactive group. As is evident from this structure, the chemically reactive groups need not be provided in the solid line portion but can be provided at any of the n or n'. In
30 this way the length of each of the 5' and 3' portions can vary by several bases (Figure 70).

In other preferred embodiments, the chemically reactive group can be, but is not limited to, $(CH_2)_nSH$; $(CH_2)_nNHR$; $(CH_2)_nX$; ribose; $COOH$; $(CH_2)_nPPh_3$; $(CH_2)_nSO_2Cl$; $(CH_2)_nCOR$; $(CH_2)_nRNH$ or $(CH_2)_nOH$, where, CH_2 can be replaced by another group which forms a linking chain (which
5 does not interfere with the terminal chemically reactive group) containing various atoms including, but not limited to CH_2 , such as methylenes, ether, ethylene glycol, thioethers, double bonds, aromatic groups and others, generally at most 20 such atoms are provided in the linking chain, most preferably only 5 - 10 atoms, and even more preferably only 3- 5 atoms; each
10 n independently is an integer from 0 to 10 inclusive and may be the same or different; each R independently is a proton or an alkyl, alkenyl (as described above) and other functional groups or conjugates such as peptides, steroids, hormones, lipids, nucleic acid sequences and others that provides nuclease resistance, improved cell association, improved cellular uptake or
15 interacellular localization. X is halogen, and Ph represents a phenyl ring.

In yet other preferred embodiments, the conditions include provision of $NaIO_4$ in contact with the ribose, and subsequent provision of a reducing group such as $NaBH_4$ or $NaCNBH_3$; or the conditions include provision of a coupling reagent.

20 In a second related aspect, the invention features a mixture of the 5' and 3' portions of the enzymatically active nucleic acids having the 3' and 5' chemically reactive groups noted above.

Those in the art will recognize that while examples are provided of half ribozymes it is possible to provide ribozymes in 3 or more portions. For
25 example, the hairpin ribozyme may be synthesized by inclusion of chemically reactive groups in helix IV and in other helices which are not critical to the enzymatic activity of the nucleic acid.

Pol III-based vectors

This invention relates to RNA polymerase III-based methods and systems
30 for expression of therapeutic RNAs in cells *in vivo* or *in vitro*.

The RNA polymerase III (pol III) promoter is one found in DNA encoding 5S, U6, adenovirus VA1, Vault, telomerase RNA, tRNA genes, etc., and is transcribed by RNA polymerase III (for a review see Geiduschek and Tocchini-Valentini, 1988 *Annu. Rev. Biochem.* 57, 873-914; Willis, 1993 *Eur. J. Biochem.* 212, 1-11). There are three major types of pol III promoters: types 1, 2 and 3 (Geiduschek and Tocchini-Valentini, 1988 *supra*; Willis, 1993 *supra*) (see Figure 1). Type 1 pol III promoter consists of three cis-acting sequence elements downstream of the transcriptional start site a) 5' sequence element (A block); b) an intermediate sequence element (I block); c) 3' sequence element (C block). 5S ribosomal RNA genes are transcribed using the type 1 pol III promoter (Specht et al., 1991 *Nucleic Acids Res.* 19, 2189-2191).

The type 2 pol III promoter is characterized by the presence of two cis-acting sequence elements downstream of the transcription start site. All Transfer RNA (tRNA), adenovirus VA RNA and Vault RNA (Kikhoefer et al., 1993, *J. Biol. Chem.* 268, 7868-7873) genes are transcribed using this promoter (Geiduschek and Tocchini-Valentini, 1988 *supra*; Willis, 1993 *supra*). The sequence composition and orientation of the two cis-acting sequence elements- A box (5' sequence element) and B box (3' sequence element) are essential for optimal transcription by RNA polymerase III.

The type 3 pol III promoter contains all of the cis-acting promoter elements upstream of the transcription start site. Upstream sequence elements include a traditional TATA box (Mataj et al., 1988 *Cell* 55, 435-442), proximal sequence element (PSE) and a distal sequence element (DSE; Gupta and Reddy, 1991 *Nucleic Acids Res.* 19, 2073-2075). Examples of genes under the control of the type 3 pol III promoter are U6 small nuclear RNA (U6 snRNA) and Telomerase RNA genes.

In addition to the three predominant types of pol III promoters described above, several other pol III promoter elements have been reported (Willis, 1993 *supra*) (see Figure 76). Epstein-Barr-virus-encoded RNAs (EBER), *Xenopus* seleno-cysteine tRNA and human 7SL RNA are examples of genes that are under the control of pol III promoters distinct from the aforementioned types of promoters. EBER genes contain a functional A and B box (similar to type 2 pol III promoter). In addition they also require an EBER-specific TATA

box and binding sites for ATF transcription factors (Howe and Shu, 1989 *Cell* 57,825-834). The seleno-cysteine tRNA gene contains a TATA box, PSE and DSE (similar to type 3 pol III promoter). Unlike most tRNA genes, the seleno-cysteine tRNA gene lacks a functional A box sequence element. It does
5 require a functional B box (Lee et al., 1989 *J. Biol. Chem.* 264, 9696-9702). The human 7SL RNA gene contains an unique sequence element downstream of the transcriptional start site. Additionally, upstream of the transcriptional start site, the 7SL gene contains binding sites for ATF class of transcription factors and a DSE (Bredow et al., 1989 *Gene* 86, 217-225).

- 10 Gilboa WO 89/11539 and Gilboa and Sullenger WO 90/13641 describe transformation of eucaryotic cells with DNA under the control of a pol III promoter. They state:

15 "In an attempt to improve antisense RNA synthesis using stable gene transfer protocols, the use of pol III promoters to drive the expression of antisense RNA can be considered. The underlying rationale for the use of pol III promoters is that they can generate substantially higher levels of RNA transcripts in cells as compared to pol II promoters. For example, it is estimated that in a eucaryotic cell there are about 6×10^7 t-RNA molecules and 7×10^5 mRNA molecules, i.e., about 100 fold more pol III transcripts of this class than total pol II transcripts. Since there are about 100 active t-RNA genes
20 per cell, each t-RNA gene will generate on the average RNA transcripts equal in number to total pol II transcripts. Since an abundant pol II gene transcript represents about 1% of total mRNA while an average pol II transcript represents about 0.01% of total mRNA, a t-RNA (pol III) based transcriptional unit may be able to generate 100 fold to 10,000 fold more RNA than a pol II based transcriptional unit. Several reports have described the
25 use of pol III promoters to express RNA in eucaryotic cells. Lewis and Manley and Sisodia have fused the Adenovirus VA-1 promoter to various DNA sequences (the herpes TK gene, globin and tubulin) and used transfection protocols to transfer the resulting DNA constructs into cultured cells which resulted in transient synthesis of RNA in the transduced cell. De la Pena and Zasloff have expressed a t-RNA-Herpes TK
30 fusion DNA construct upon microinjection into frog oocytes. Jennings and Molloy have constructed an antisense RNA template by fusing the VA-1 gene promoter to a DNA fragment derived from SV40 based vector which also resulted in transient expression of antisense RNA and limited inhibition of the target gene". [Citations omitted.]

The authors describe a fusion product of a chimeric tRNA and an RNA product (see Fig. 1C of WO 90/13641). In particular they describe a human tRNA^{met} derivative 3-5. 3-5 was derived from a cloned human tRNA gene by deleting 19 nucleotides from the 3' end of the gene. The authors indicate that
5 the truncated gene can be transcribed if a termination signal is provided, but that no processing of the 3' end of the RNA transcript takes place.

Adeniyi-Jones et al., 1984 *Nucleic Acids Res.* 12, 1101-1115, describe certain constructions which "may serve as the basis for utilizing the tRNA gene as a 'portable promoter' in engineered genetic constructions." The authors
10 describe the production of a so-called $\Delta 3'-5$ in which 11 nucleotides of the 3'-end of the mature tRNA^{met} sequence are replaced by a plasmid sequence, and are not processed to generate a mature tRNA. The authors state:

"the properties of the tRNA^{met} 3' deletion plasmids described in this study suggest their potential use in certain engineered genetic constructions. The tRNA gene could
15 be used to promote transcription of theoretically any DNA sequence fused to the 3' border of the gene, generating a fusion gene which would utilize the efficient polymerase III promoter of the human tRNA^{met} gene. By fusion of the DNA sequence to a tRNA^{met} deletion mutant such as $\Delta 3'-4$, a long read-through transcript would be generated in vivo (dependent, of course, on the absence of effective RNA polymerase
20 III termination sequences). Fusion of the DNA sequence to a tRNA^{met} deletion mutant such as $\Delta 3'-5$ would lead to the generation of a co-transcript from which subsequent processing of the tRNA leader at the 5' portion of the fused transcript would be blocked. Control over processing may be of some biological use in engineered constructions, as suggested by properties of mRNA species bearing tRNA sequences as 5' leaders in
25 prokaryotes. Such "dual transcripts" code for several predominant bacterial proteins such as EF-Tu and may use the tRNA leaders as a means of stabilizing the transcript from degradation in vivo. The potential use of the tRNA^{met} gene as a "promoter leader" in eukaryotic systems has been realized recently in our laboratory. Fusion genes consisting of the deleted tRNA^{met} sequences contained on plasmids $\Delta 3'-4$
30 and $\Delta 3'-5$ in front of a promoter-less Herpes simplex type I thymidine kinase gene yield viral-specific enzyme resulting from RNA polymerase III dependent transcription in both *X. laevis* oocytes and somatic cells". [References omitted].

Sullenger et al., 1990 *Cell* 63, 601-619, describe over-expression of *TAR*-containing sequences using a chimeric tRNA^{met}-*TAR* transcription unit in a double copy (DC) murine retroviral vector.

5 Sullenger et al., 1990 *Molecular and Cellular Bio.* 10, 6512, describe expression of chimeric tRNA driven antisense transcripts. It indicates:

10 "successful use of a tRNA-driven antisense RNA transcription system was dependent on the use of a particular type of retroviral vector, the double-copy (DC) vector, in which the chimeric tRNA gene was inserted in the viral LTR. The use of an RNA pol III-based transcription system to stably express high levels of foreign RNA sequences in cells may have other important applications. Foremost, it may significantly improve the ability to inhibit endogenous genes in eucaryotic cells for the study of gene expression and function, whether antisense RNA, ribozymes, or competitors of sequence-specific binding factors are used. tRNA-driven transcription systems may be particularly useful for introducing "mutations" into the germ line, i.e., for generating transgenic animals or transgenic plants. Since tRNA genes are ubiquitously expressed in all cell types, the chimeric tRNA genes may be properly expressed in all tissues of the animal, in contrast to the more idiosyncratic behavior of RNA pol II-based transcription units. However, homologous recombination represents a more elegant although, at present, very cumbersome approach for introducing mutations into the germ line. In either case, the ability to generate transgenic animals or plants carrying defined mutations will be an extremely valuable experimental tool for studying gene function in a developmental context and for generating animal models for human genetic disorders. In addition, tRNA-driven gene inhibition strategies may also be useful in creating pathogen-resistant livestock and plants. [References omitted.]

25 Cotten and Bimstiel, 1989 *EMBO Jml.* 8, 3861, describe the use of tRNA genes to increase intracellular levels of ribozymes. The authors indicate that the ribozyme coding sequences were placed between the A and the B box internal promoter sequences of the *Xenopus* tRNA^{met} gene. They also indicate that the targeted hammerhead ribozymes were active *in vivo*.

30 Yu et al., 1993 *Proc. Natl. Acad. Sci. USA* 90, 5340, describe the use of a VAI promoter to express a hairpin ribozyme. The resulting transcript consisted

of the first 104 nucleotides of the VAI RNA, followed by the ribozyme sequence and the terminator sequence.

Lieber and Strauss, 1995 *Mol. Cellular Bio.* 15, 540, inserted a hammerhead ribozyme sequence in the central domain of a VAI RNA.

- 5 Pol III-based vectors are described in Stinchcomb et al., WO 95/23225. Another example is provided below.

Example 1: Stromelysin Hammerhead ribozymes

- By engineering ribozyme motifs applicant has designed several ribozymes directed against stromelysin mRNA sequences. These ribozymes
10 are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave stromelysin target sequences *in vitro* is evaluated.

- The ribozymes are tested for function *in vivo* by analyzing stromelysin expression levels. Ribozymes are delivered to cells by incorporation into
15 liposomes, by complexing with cationic lipids, by microinjection, and/or by expression from DNA/RNA vectors. Stromelysin expression is monitored by biological assays, ELISA, by indirect immunofluorescence, and/or by FACS analysis. Stromelysin mRNA levels are assessed by Northern analysis, RNase protection, primer extension analysis and/or quantitative RT-PCR.
20 Ribozymes that block the induction of stromelysin activity and/or stromelysin mRNA by more than 50% are identified.

- Ribozymes targeting selected regions of mRNA associated with arthritic disease are chosen to cleave the target RNA in a manner which preferably inhibits translation of the RNA. Genes are selected such that inhibition of
25 translation will preferably inhibit cell replication, e.g., by inhibiting production of a necessary protein or prevent production of an undesired protein, e.g., stromelysin. Selection of effective target sites within these critical regions of mRNA may entail testing the accessibility of the target RNA to hybridization with various oligonucleotide probes. These studies can be performed using
30 RNA or DNA probes and assaying accessibility by cleaving the hybrid molecule with RNaseH (see below). Alternatively, such a study can use

ribozyme probes designed from secondary structure predictions of the mRNAs, and assaying cleavage products by polyacrylamide gel electrophoresis (PAGE), to detect the presence of cleaved and uncleaved molecules.

- 5 In addition, potential ribozyme target sites within the rabbit stromelysin mRNA sequence (1795 nucleotides) were located and aligned with the human target sites. Because the rabbit stromelysin mRNA sequence has an 84% sequence identity with the human sequence, many ribozyme target sites are also homologous. Thus, the rabbit has potential as an appropriate animal
10 model in which to test ribozymes that are targeted to human stromelysin but have homologous or nearly homologous cleavage sites on rabbit stromelysin mRNA as well (Tables AII-AVI, AVIII & AIX). Thirty of the 316 UH sites in the rabbit sequence are identical with the corresponding site in the human sequence with respect to at least 14 nucleotides surrounding the potential
15 ribozyme cleavage sites. The nucleotide in the RNA substrate that is immediately adjacent (5') to the cleavage site is unpaired in the ribozyme-substrate complex (see Fig. 1) and is consequently not included in the comparison of human and rabbit potential ribozyme sites. In choosing human ribozyme target sites for continued testing, the presence of identical or nearly
20 identical sites in the rabbit sequence is considered.

Example 2: Superior sites

- Potential ribozyme target sites were subjected to further analysis using computer folding programs (Mfold or a Macintosh-based version of the following program, LRNA (Zucker (1989) Science 244:48), to determine if 1)
25 the target site is substantially single-stranded and therefore predicted to be available for interaction with a ribozyme, 2) if a ribozyme designed to that site is predicted to form stem II but is generally devoid of any other intramolecular base pairing, and 3) if the potential ribozyme and the sequence flanking both sides of the cleavage site together are predicted to interact correctly. The
30 sequence of Stem II can be altered to maintain a stem at that position but minimize intramolecular basepairing with the ribozyme's substrate binding arms. Based on these minimal criteria, and including all the sites that are identical in human and rabbit stromelysin mRNA sequence, a subset of 66

potential superior ribozyme target sites was chosen (as first round targets) for continued analysis. These are SEQ. ID. NOS.: 34, 35, 37, 47, 54, 57, 61, 63, 64, 66, 76, 77, 79, 87, 88, 96, 97, 98, 99, 100, 107, 110, 121, 126, 128, 129, 133, 140, 146, 148, 151, 162, 170, 179, 188, 192, 194, 196, 199, 202, 203, 207, 208, 218, 220, 223, 224, 225, 227, 230, 232, 236, 240, 245, 246, 256, 259, 260, 269, 280, 281, 290, 302, 328, 335 and 353 (see Table AIII).

Example 3: Accessible sites

To determine if any or all of these potential superior sites might be accessible to a ribozyme directed to that site, an RNase H assay is carried out. Using this assay, the accessibility of a potential ribozyme target site to a DNA oligonucleotide probe can be assessed without having to synthesize a ribozyme to that particular site. If the complementary DNA oligonucleotide is able to hybridize to the potential ribozyme target site then RNase H, which has the ability to cleave the RNA of a DNA/RNA hybrid, will be able to cleave the target RNA at that particular site. Specific cleavage of the target RNA by RNase H is an indication that that site is "open" or "accessible" to oligonucleotide binding and thus predicts that the site will also be open for ribozyme binding. By comparing the relative amount of specific RNase H cleavage products that are generated for each DNA oligonucleotide/site, potential ribozyme sites can be ranked according to accessibility.

To analyze target sites using the RNase H assay, DNA oligonucleotides (generally 13-15 nucleotides in length) that are complementary to the potential target sites are synthesized. Body-labeled substrate RNAs (either full-length RNAs or ~500-600 nucleotide subfragments of the entire RNA) are prepared by *in vitro* transcription in the presence of a ³²P-labeled nucleotide. Unincorporated nucleotides are removed from the ³²P-labeled substrate RNA by spin chromatography on a G-50 Sephadex column and used without further purification. To carry out the assay, the ³²P-labeled substrate RNA is pre-incubated with the specific DNA oligonucleotide (1 μM and 0.1 μM final concentration) in 20 mM Tris-HCl, pH 7.9, 100 mM KCl, 10 mM MgCl₂, 0.1 mM EDTA, 0.1 mM DTT at 37°C for 5 minutes. An excess of RNase H (0.8 units/10 μl reaction) is added and the incubation is continued for 10 minutes. The reaction is quenched by the addition of an equal volume of 95% formamide.

20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol FF after which the sample is heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. RNase H-cleaved RNA products are separated from uncleaved RNA on denaturing polyacrylamide gels, visualized
5 by autoradiography and the amount of cleavage product is quantified.

RNase H analysis on the 66 potential ribozyme sites (round 1) was carried out and those DNA oligonucleotides/sites that supported the most RNase H cleavage were determined. These assays were carried out using full-length human and rabbit stromelysin RNA as substrates. Results
10 determined on human stromelysin RNA indicated that 23 of the 66 sites supported a high level of RNase H cleavage, and an additional 13 supported a moderate level of RNase H cleavage. Twenty-two sites were chosen from among these two groups for continued study. Two of the criteria used for making this choice were 1) that the particular site supported at least moderate
15 RNase H cleavage on human stromelysin RNA and 2) that the site have two or fewer nucleotide differences between the rabbit and the human stromelysin sequence. RNase H accessibility on rabbit stromelysin RNA was determined, but was not used as a specific criteria for these choices. Those DNA oligonucleotides that are not totally complementary to the rabbit sequence
20 may not be good indicators of the relative amount of RNase H cleavage, possibly because the mismatch leads to less efficient hybridization of the DNA oligonucleotide to the mismatched RNA substrate and therefore less RNase H cleavage is seen.

Example 4: Analysis of Ribozymes

25 Ribozymes were then synthesized to 22 sites (Table AV) predicted to be accessible as judged the RNase H assay. Eleven of these 22 sites are identical to the corresponding rabbit sites. The 22 sites are SEQ. ID, NOS.: 34, 35, 57, 125, 126, 127, 128, 129, 140, 162, 170, 179, 188, 223, 224, 236, 245, 246, 256, 259, 260, 281. The 22 ribozymes were chemically synthesized
30 with recognition arms of either 7 nucleotides or 8 nucleotides, depending on which ribozyme alone and ribozyme-substrate combinations were predicted by the computer folding program (Mfold) to fold most correctly. After synthesis, ribozymes are either purified by HPLC or gel purified.

These 22 ribozymes were then tested for their ability to cleave both human and rabbit full-length stromelysin RNA. Full-length, body-labeled stromelysin RNA is prepared by *in vitro* transcription in the presence of [α - 32 P]CTP, passed over a G 50 Sephadex column by spin chromatography and used as substrate RNA without further purification. Assays are performed by prewarming a 2X concentration of purified ribozyme in ribozyme cleavage buffer (50 mM Tris-HCl, pH 7.5 at 37°C, 10 mM MgCl₂) and the cleavage reaction is initiated by adding the 2X ribozyme mix to an equal volume of substrate RNA (maximum of 1-5 nM) that has also been prewarmed in cleavage buffer. As an initial screen, assays are carried out for 1 hour at 37°C using a final concentration of 1 μ M and 0.1 μ M ribozyme, *i.e.*, ribozyme excess. The reaction is quenched by the addition of an equal volume of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol FF after which the sample is heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. Full-length substrate RNA and the specific RNA products generated by ribozyme cleavage are visualized on an autoradiograph of the gel.

Of the 22 ribozymes tested, 21 were able to cleave human and rabbit substrate RNA *in vitro* in a site-specific manner. In all cases, RNA cleavage products of the appropriate lengths were visualized. The size of the RNA was judged by comparison to molecular weight standards electrophoresed in adjacent lanes of the gel. The fraction of substrate RNA cleaved during a ribozyme reaction can be used as an assessment of the activity of that ribozyme *in vitro*. The activity of these 22 ribozymes on full-length substrate RNA ranged from approximately 10% to greater than 95% of the substrate RNA cleaved in the ribozyme cleavage assay using 1 μ M ribozyme as described above. A subset of seven of these ribozymes was chosen for continued study. These seven ribozymes (denoted in Table AV) were among those with the highest activity on both human and rabbit stromelysin RNA. Five of these seven sites have sequence identity between human and rabbit stromelysin RNAs for a minimum of 7 nucleotides in both directions flanking the cleavage site. These sites are 883, 947, 1132, 1221 and 1410. and the ribozymes are SEQ. ID. NOS.: 368, 369, 370, 371, 372, 373, and 374.

Example 5: Arm Length Tests

In order to test the effect of arm length variations on the cleavage activity of a ribozyme to a particular site *in vitro*, ribozymes to these seven sites were designed that had alterations in the binding arm lengths. For each site, a complete set of ribozymes was synthesized that included ribozymes with binding arms of 6 nucleotides, 7 nucleotides, 8 nucleotides, 10 nucleotides and 12 nucleotides, *i.e.*, 5 ribozymes to each site. These ribozymes were gel-purified after synthesis and tested in ribozyme cleavage assays as described above.

After analysis of the 35 ribozymes, five ribozymes with varied arm lengths to each of these seven sites, it was clear that two ribozymes were the most active *in vitro*. These two ribozymes had seven nucleotide arms directed against human sequence cleavage sites of nucleotide 617 and nucleotide 820. These are referred to as RZ 617H 7/7 and RZ 820H 7/7 denoting the human (H) sequence cleavage site (617 or 820) and the arm length on the 5' and 3' side of the ribozyme molecule.

Example : Testing the efficacy of ribozymes in cell culture

The two most active ribozymes *in vitro* (RZ 617H 7/7 and RZ 820H 7/7) were then tested for their ability to cleave stromelysin mRNA in the cell. Primary cultures of human or rabbit synovial fibroblasts were used in these experiments. For these efficacy tests, ribozymes with 7 nucleotide arms were synthesized with 2' O- methyl modifications on the 5 nucleotides at the 5' end of the molecule and on the 5 nucleotides at the 3' end of the molecule. For comparison, ribozymes to the same sites but with 12 nucleotide arms (RZ 617H 12/12 and RZ 820H 12/12) were also synthesized with the 2' O methyl modifications at the 5 positions at the end of both binding arms. Inactive ribozymes that contain 2 nucleotide changes in the catalytic core region were also prepared for use as controls. The catalytic core in the inactive ribozymes is C U U A U G A G G C C G A A A G G C C G A U versus CUGAUGAGGCCGAAAGGCCGAA in the active ribozymes. The inactive ribozymes show no cleavage activity *in vitro* when measured on full-length RNA in the typical ribozyme cleavage assay at a 1 μ M concentration for 1 hour.

The general assay was as follows: Fibroblasts, which produce stromelysin, are serum-starved overnight and ribozymes or controls are offered to the cells the next day. Cells are maintained in serum-free media. The ribozyme can be applied to the cells as free ribozyme, or in association with various delivery vehicles such as cationic lipids (including Transfectam™, Lipofectin™ and Lipofectamine™), conventional liposomes, non-phospholipid liposomes or biodegradable polymers. At the time of ribozyme addition, or up to 3 hours later, Interleukin-1 α (typically 20 units/ml) can be added to the cells to induce a large increase in stromelysin expression. The production of stromelysin can then be monitored over a time course, usually up to 24 hours.

If a ribozyme is effective in cleaving stromelysin mRNA within a cell, the amount of stromelysin mRNA will be decreased or eliminated. A decrease in the level of cellular stromelysin mRNA, as well as the appearance of the RNA products generated by ribozyme cleavage of the full-length stromelysin mRNA, can be analyzed by methods such as Northern blot analysis, RNase protection assays and/or primer extension assays. The effect of ribozyme cleavage of cellular stromelysin mRNA on the production of the stromelysin protein can also be measured by a number of assays. These include the ELISA (Enzyme-Linked Immuno Sorbent Assay) and an immunofluorescence assay described below. In addition, functional assays have been published that monitor stromelysin's enzymatic activity by measuring degradation of its primary substrate, proteoglycan.

Example 7: Analysis of Stromelysin Protein

Stromelysin secreted into the media of Interleukin-1 α -induced human synovial fibroblasts was measured by ELISA using an antibody that recognizes human stromelysin. Where present, a Transfectam™-ribozyme complex (0.15 μ M ribozyme final concentration) was offered to 2-4 x 10⁵ serum-starved cells for 3 hours prior to induction with Interleukin-1 α . The Transfectam™ was prepared according to the manufacturer (Promega Corp.) except that 1:1 (w/w) dioleoyl phosphatidylethanolamine was included. The Transfectam™-ribozyme complex was prepared in a 5:1 charge ratio. Media was harvested 24 hours after the addition of Interleukin-1 α . The control (NO RZ) is Transfectam™ alone applied to the cell. Inactive ribozymes, with 7

nucleotide arms or 12 nucleotide arms have the two inactivating changes to the catalytic core that are described above. Cell samples were prepared in duplicate and the assay was carried out on several dilutions of the conditioned media from each sample. Results of the ELISA are presented below as a percent of stromelysin present vs. the control (NO RZ) which is set at 100%.

		<u>RZ TARGET SITE</u>	
<u>TREATMENT</u>		<u>617H</u>	<u>820H</u>
	RZ 7/7	06.83	07.05
	RZ 12/12	18.47	33.90
10	INACTIVE RZ 7/7	100	100
	INACTIVE RZ 12/12	100	100
	NO RZ CONTROL	100	100

The results above clearly indicate that treatment with active ribozyme, either RZ 617H 7/7 and RZ 820H 7/7, has a dramatic effect on the amount of stromelysin secreted by the cells. When compared to untreated, control cells or cells treated with inactive ribozymes, the level of stromelysin was decreased by approximately 93%. Ribozymes to the same sites, but synthesized with 12 nucleotide binding arms, were also efficacious, causing a decrease in stromelysin to ~66 to ~81% of the control. In previous *in vitro* ribozyme cleavage assays, RZ 617H 7/7 and RZ 820H 7/7 had better cleavage activity on full-length RNA substrates than ribozymes with 12 nucleotide arms directed to the same sites (617H 12/12 and RZ 820H 12/12).

25 Example 8: Immunofluorescent Assay

An alternative method of stromelysin detection is to visualize stromelysin protein in the cells by immunofluorescence. For this assay, cells are treated

with monensin to prevent protein secretion from the cell. The stromelysin retained by the cells after monensin addition can then be visualized by immunofluorescence using either conventional or confocal microscopy. Generally, cells were serum-starved overnight and treated with ribozyme the following day for several hours. Monensin was then added and after ~5-6 hours, monensin-treated cells were fixed and permeabilized by standard methods and incubated with an antibody recognizing human stromelysin. Following an additional incubation period with a secondary antibody that is conjugated to a fluorophore, the cells were observed by microscopy. A decrease in the amount of fluorescence in ribozyme-treated cells, compared to cells treated with inactive ribozymes or media alone, indicates that the level of stromelysin protein has been decreased due to ribozyme treatment.

As visualized by the immunofluorescence technique described above, treatment of human synovial fibroblasts with either RZ 617H 7/7 or RZ 820H 7/7 (final concentrations of 1.5 μ M free ribozyme or 0.15 μ M ribozyme complexed with Transfectam™ resulted in a significant decrease in fluorescence, and therefore stromelysin protein, when compared with controls. Controls consisted of treating with media or Transfectam™ alone. Treatment of the cells with the corresponding inactive ribozymes with two inactivating changes in the catalytic core resulted in immunofluorescence similar to the controls without ribozyme treatment.

Rabbit synovial fibroblasts were also treated with RZ 617H 7/7 or RZ 820H 7/7, as well as with the two corresponding ribozymes (RZ 617R 7/7 or RZ 820R 7/7) that each have the appropriate one nucleotide change to make them completely complementary to the rabbit target sequence. Relative to controls that had no ribozyme treatment, immunofluorescence in Interleukin-1 α -induced rabbit synovial fibroblasts was visibly decreased by treatment with these four ribozymes, whether specific for rabbit or human mRNA sequence. For the immunofluorescence study in rabbit synovial fibroblasts, the antibody to human stromelysin was used.

Example 9: Ribozyme Cleavage of Cellular RNA

The following method was used in this example.

Primer extension assay:

The primer extension assay was used to detect full-length RNA as well as the 3' ribozyme cleavage products of the RNA of interest. The method involves synthesizing a DNA primer (generally ~20 nucleotides in length) that can hybridize to a position on the RNA that is downstream (3') of the putative ribozyme cleavage site. Before use, the primer was labeled at the 5' end with ^{32}P [ATP] using T4 polynucleotide kinase and purified from a gel. The labeled primer was then incubated with a population of nucleic acid isolated from a cellular lysate by standard procedures. The reaction buffer was 50 mM Tris-HCl, pH 8.3, 3 mM MgCl_2 , 20 mM KCl, and 10 mM DTT. A 30 minute extension reaction follows, in which all DNA primers that have hybridized to the RNA were substrates for reverse transcriptase, an enzyme that will add nucleotides to the 3' end of the DNA primer using the RNA as a template. Reverse transcriptase was obtained from Life Technologies and is used essentially as suggested by the manufacturer. Optimally, reverse transcriptase will extend the DNA primer, forming cDNA, until the end of the RNA substrate is reached. Thus, for ribozyme-cleaved RNA substrates, the cDNA product will be shorter than the resulting cDNA product of a full-length, or uncleaved RNA substrate. The differences in size of the ^{32}P -labeled cDNAs produced by extension can then be discriminated by electrophoresis on a denaturing polyacrylamide gel and visualized by autoradiography.

Strong secondary structure in the RNA substrate can, however, lead to premature stops by reverse transcriptase. This background of shorter cDNAs is generally not a problem unless one of these prematurely terminated products electrophoreses in the expected position of the ribozyme-cleavage product of interest. Thus, 3' cleavage products are easily identified based on their expected size and their absence from control lanes. Strong stops due to secondary structure in the RNA do, however, cause problems in trying to quantify the total full-length and cleaved RNA present. For this reason, only the relative amount of cleavage can easily be determined.

The primer extension assay was carried out on RNA isolated from cells that had been treated with TransfectamTM-complexed RZ 617H 7/7, RZ 820H 7/7, RZ 617H 12/12 and RZ 820H 12/12. Control cells had been treated with

Transfectam™ alone. Primer extensions on RNA from cells treated with the Transfectam™ complexes of the inactive versions of these four ribozymes were also prepared. The 20 nucleotide primer sequence is 5' AATGAAAACGAGGTCCTTGC 3' and it is complementary to a region about 285 nucleotides downstream of ribozyme site 820. For ribozymes to site 617, the cDNA length for the 3' cleavage product is 488 nucleotides, for 820 the cDNA product is 285 nucleotides. Full-length cDNA will be 1105 nucleotides in length. Where present, 1 ml of 0.15 µM ribozyme was offered to ~2-3 x 10⁵ serum-starved human synovial fibroblasts. After 3 hours, 20 units/ml Interleukin-1α was added to the cells and the incubation continued for 24 hours.

³²P-labeled cDNAs of the correct sizes for the 3' products were clearly visible in lanes that contained RNA from cells that had been treated with active ribozymes to sites 617 and 820. Ribozymes with 7 nucleotide arms were judged to be more active than ribozymes with 12 nucleotide arms by comparison of the relative amount of 3' cleavage product visible. This correlates well with the data obtained by ELISA analysis of the conditioned media from these same samples. In addition, no cDNAs corresponding to the 3' cleavage products were visible following treatment of the cells with any of the inactive ribozymes.

To insure that ribozyme cleavage of the RNA substrate was not occurring during the preparation of the cellular RNA or during the primer extension reaction itself, several controls have been carried out. One control was to add body-labeled stromelysin RNA, prepared by *in vitro* transcription, to the cellular lysate. This lysate was then subjected to the typical RNA preparation and primer extension analysis except that non-radioactive primer was used. If ribozymes that are present in the cell at the time of cell lysis are active under any of the conditions during the subsequent analysis, the added, body-labeled stromelysin RNA will become cleaved. This, however, is not the case. Only full-length RNA was visible by gel analysis, no ribozyme cleavage products were present. This is evidence that the cleavage products detected in RNA from ribozyme-treated cells resulted from ribozyme cleavage in the cell, and not during the subsequent analysis.

Example 10: RNase Protection Assay

By RNase protection analysis, both the 3' and the 5' products generated by ribozyme cleavage of the substrate RNA in a cell can be identified. The RNase protection assay is carried out essentially as described in the protocol provided with the Lysate Ribonuclease Protection Kit (United States Biochemical Corp.) The probe for RNase protection is an RNA that is complementary to the sequence surrounding the ribozyme cleavage site. This "antisense" probe RNA is transcribed *in vitro* from a template prepared by the polymerase chain reaction in which the 5' primer was a DNA oligonucleotide containing the T7 promoter sequence. The probe RNA is body labeled during transcription by including ^{32}P [CTP] in the reaction and purified away from unincorporated nucleotide triphosphates by chromatography on G-50 Sephadex. The probe RNA (100,000 to 250,000 cpm) is allowed to hybridize overnight at 37°C to the RNA from a cellular lysate or to RNA purified from a cell lysate. After hybridization, RNase T₁ and RNase A are added to degrade all single-stranded RNA and the resulting products are analyzed by gel electrophoresis and autoradiography. By this analysis, full-length, uncleaved target RNAs will protect the full-length probe. For ribozyme-cleaved target RNAs, only a portion of the probe will be protected from RNase digestion because the cleavage event has occurred in the region to which the probe binds. This results in two protected probe fragments whose size reflects the position at which ribozyme cleavage occurs and whose sizes add up to the size of the full-length protected probe.

RNase protection analysis was carried out on cellular RNA isolated from rabbit synovial fibroblasts that had been treated either with active or inactive ribozyme. The ribozymes tested had 7 nucleotide arms specific to the rabbit sequence but corresponding to human ribozyme sites 617 and 820 (i.e. RZ 617R 7/7, RZ 820R 7/7). The inactive ribozymes to the same sites also had 7 nucleotide arms and included the two inactivating changes described above. The inactive ribozymes were not active on full-length rabbit stromelysin RNA in a typical 1 hour ribozyme cleavage reaction *in vitro* at a concentration of 1 μM . For all samples, one ml of 0.15 μM ribozyme was administered as a Transfectam™ complex to serum-starved cells. Addition of Interleukin-1 α followed 3 hours later and cells were harvested after 24 hours. For samples

from cells treated with either active ribozyme tested, the appropriately-sized probe fragments representing ribozyme cleavage products were visible. For site 617, two fragments corresponding to 125 and 297 nucleotides were present, for site 820 the two fragments were 328 and 94 nucleotides in length.

- 5 No protected probe fragments representing RNA cleavage products were visible in RNA samples from cells that not been treated with any ribozyme, or in cells that had received the inactive ribozymes. Full-length protected probe (422 nucleotides in length) was however visible, indicating the presence of full-length, uncleaved stromelysin RNA in these samples.

10 Delivery of Free and Transfectam-Complexed Ribozymes to Fibroblasts

- Ribozymes can be delivered to fibroblasts complexed to a cationic lipid or in free form. To deliver free ribozyme, an appropriate dilution of stock ribozyme (final concentration is usually 1.5 μ M) is made in serum-free medium; if a radioactive tracer is to be used (i.e., 32 P), the specific activity of the ribozyme is adjusted to 800-1200 cpm/pmol. To deliver ribozyme complexed with the cationic lipid Transfectam, the lipid is first prepared as a stock solution containing 1/1 (w/w) dioleoylphosphatidylcholine (DOPE). Ribozyme is mixed with the Transfectam/DOPE mixture at a 1/5 (RZ/TF) charge ratio; for a 36-mer ribozyme, this is a 45-fold molar excess of Transfectam (Transfectam has 4 positive charges per molecule). After a 10 min incubation at room temperature, the mixture is diluted and applied to cells, generally at a ribozyme concentration of 0.15 μ M. For 32 P experiments, the specific activity of the ribozyme is the same as for the free ribozyme experiments.

- After 24 hour, about 30% of the offered Transfectam-ribozyme cpm's are cell-associated (in a nuclease-resistant manner). Of this, about 10-15% of the cpm's represent intact ribozyme; this is about 20-25 million ribozymes per cell. For the free ribozyme, about 0.6% of the offered dose is cell-associated after 24 hours. Of this, about 10-15% is intact; this is about 0.6-0.8 million ribozymes per cell.

30 Example 11: *In vitro* cleavage of stromelysin mRNA by HH ribozymes

In order to screen for additional HH ribozyme cleavage sites, ribozymes, targeted against some of the sites listed in example 2 and Table 3, were

synthesized. These ribozymes were extensively modified such that: 5' terminal nucleotides contain phosphorothioate substitutions; except for five ribose residues in the catalytic core, all the other 2'-hydroxyl groups within the ribozyme were substituted with either 2'-O-methyl groups or 2'-C-allyl modifications. The aforementioned modifications are meant to be non-limiting modifications. Those skilled in the art will recognize that other embodiments can be readily generated using the techniques known in the art.

These ribozymes were tested for their ability to cleave RNA substrates *in vitro*. Referring to Fig. 7, *in vitro* RNA cleavage by HH ribozymes targeted to sites 21, 463, 1049, 1366, 1403, 1410 and 1489 (SEQ. ID. NOS. 35, 98, 202, 263, 279, 281 and 292 respectively) was assayed at 37°C. Substrate RNAs were 5' end-labeled using [γ -³²P]ATP and T4 polynucleotide kinase enzyme. In a standard cleavage reaction under "ribozyme excess" conditions, ~1 nM substrate RNA and 40 nM ribozyme were denatured separately by heating to 90°C for 2 min followed by snap cooling on ice for 10 min. The substrate and the ribozyme reaction mixtures were renatured in a buffer containing 50 mM Tris-HCl, pH 7.5 and 10 mM MgCl₂ at 37°C for 10 min. Cleavage reaction was initiated by mixing the ribozyme and the substrate RNA and incubating at 37°C. Aliquots of 5 μ l were taken at regular intervals of time and the reaction quenched by mixing with an equal volume of formamide stop mix. The samples were resolved on a 20% polyacrylamide/urea gel.

A plot of percent RNA substrate cleaved as a function of time is shown in Fig. 7. The plot shows that all six HH ribozymes cleaved the target RNA efficiently. Some HH ribozymes were, however, more efficient than others (e.g., 1049HH cleaves faster than 1366HH).

Ribozyme Efficacy Assay in Cultured HS-27 Cells (Used in the Following Examples):

Ribozymes were assayed on either human foreskin fibroblasts(HS-27) cell line or primary human synovial fibroblasts (HSF). All cells were plated the day before the assay in media containing 10% fetal bovine serum in 24 well plates at a density of 5×10^4 cells/well. At 24 hours after plating, the media was removed from the wells and the monolayers were washed with Dulbeccos

phosphate buffered saline (PBS). The cells were serum starved for 24 h by incubating the cells in media containing 0.5% fetal bovine serum (FBS; 1 ml/well). Ribozyme/lipid complexes were prepared as follows: Ribozymes and LipofectAMINE were diluted separately in serum-free DMEM plus 20 mM Hepes pH 7.3 to 2X final concentration, then equal volumes were combined, vortexed and incubated at 37°C for 15 minutes. The charge ratio of LipofectAmine: ribozyme was 3:1. Cells were washed twice with PBS containing Ca^{2+} and Mg^{2+} . Cells were then treated the ribozyme/lipid complexes and incubated at 37°C for 1.5 hours. FBS was then added to a final concentration of 10%. Two hours after FBS addition, the ribozyme containing solution was removed and 0.5 ml DMEM containing 50 u/ml IL-1, 10% FBS, 20 mM Hepes pH 7.3 added. Supernatants were harvested 16 hours after IL-1 induction and assayed for stromelysin expression by ELISA. Polyclonal antibody against Matrix Metalloproteinase 3 (Biogenesis, NH) was used as the detecting antibody and anti-stromelysin monoclonal antibody was used as the capturing antibody in the sandwich ELISA (Maniatis et al., *supra*) to measure stromelysin expression.

Example 12: Ribozyme-Mediated Inhibition of Stromelysin Expression in human fibroblast cells

Referring to Figs. 8 through 13, HH ribozymes, targeted to sites 21, 463, 1049, 1366, 1403, 1410 and 1489 within human stromelysin-1 mRNA, were transfected into HS-27 fibroblast or HSF cell line as described above. Catalytically inactive ribozymes that contain 2 nucleotide changes in the catalytic core region were also synthesized for use as controls. The catalytic core in the inactive ribozymes was CUUAUGAGGCCGAAAGGCCGAU versus CUGAUGAGGCCGAAAGGCCGAA in the active ribozymes. The inactive ribozymes show no cleavage activity *in vitro* when measured on full-length RNA in the typical ribozyme cleavage assay at a 1 μM concentration for 1 hour. Levels of stromelysin protein were measured using a sensitive ELISA protocol as described above. + IL-1 in the figures mean that cells were treated with IL-1 to induce the expression of stromelysin expression. -IL-1 means that the cells were not treated. Figs. 8 through 13 show the dramatic reduction in the levels of stromelysin protein expressed in cells that were transfected with active HH ribozymes. This decrease in the level of

stromelysin production is over and above some non-specific inhibition seen in cells that were transfected with catalytically inactive ribozymes. There is on an average a greater than 50% inhibition in stromelysin production (in cells transfected with active HH ribozymes) when compared with control cells that were transfected with inactive ribozymes. These results suggest that the reduction in stromelysin production in HS-27 cells is mediated by sequence-specific cleavage of human stromelysin-1 mRNA by catalytically active HH ribozymes. Reduction in stromelysin protein production in cells transfected with catalytically inactive ribozymes may be due to some "antisense effect" caused by binding of the inactive ribozyme to the target RNA and physically preventing translation.

Example 13: Ribozyme-mediated inhibition of stromelysin expression in Rabbit Knee

In order to extend the ribozyme efficacy in cell culture, applicant has chosen to use rabbit knee as a reasonable animal model to study ribozyme-mediated inhibition of rabbit stromelysin protein expression. Applicant selected a HH ribozyme (1049HH), targeted to site 1049 within human stromelysin-1 mRNA, for animal studies because site 1049 is 100% identical to site 1060 (Tables AIII and AVI) within rabbit stromelysin mRNA. This has enabled applicant to compare the efficacy of the same ribozyme in human as well as in rabbit systems.

Male New Zealand White Rabbits (3-4 Kg) were anaesthetized with ketamine-HCl/xylazine and injected intra-articularly (I.T.) in both knees with 100 µg ribozyme (e.g., SEQ. ID. NO. 202) in 0.5 ml phosphate buffered saline (PBS) or PBS alone (Controls). The IL-1 (human recombinant IL-1 α , 25 ng) was administered I.T., 24 hours following the ribozyme administration. Each rabbit received IL-1 in one knee and PBS alone in the other. The synovium was harvested 6 hours post IL-1 infusion, snap frozen in liquid nitrogen, and stored at -80°C. Total RNA is extracted with TRIzol reagent (GIBCO BRL, Gaithersburg, MD), and was analyzed by Northern-blot analysis and/or RNase-protection assay. Briefly, 0.5 µg cellular RNA was separated on 1.0 % agarose/formaldehyde gel and transferred to Zeta-Probe GT nylon membrane (Bio-Rad, Hercules, CA) by capillary transfer for ~16 hours. The blots were

baked for two hours and then pre-hybridized for 2 hours at 65°C in 10 ml Church hybridization buffer (7 % SDS, 500 mM phosphate, 1 mM EDTA, 1% Bovine Serum Albumin). The blots were hybridized at 65°C for ~16 hours with 10⁶ cpm/ml of full length ³²P-labeled complementary RNA (cRNA) probes to rabbit stromelysin mRNA (cRNA added to the pre-hybridization buffer along with 100 µl 10mg/ml salmon sperm DNA). The blot was rinsed once with 5% SDS, 25 mM phosphate, 1 mM EDTA and 0.5% BSA for 10 min at room temperature. This was followed by two washes (10 min each wash) with the same buffer at 65°C, which was then followed by two washes (10 min each wash) at 65°C with 1% SDS, 25 mM phosphate and 1 mM EDTA. The blot was autoradiographed. The blot was reprobbed with a 100 nt cRNA probe to 18S rRNA as described above. Following autoradiography, the stromelysin expression was quantified on a scanning densitometer, which is followed by normalization of the data to the 18S rRNA band intensities.

As shown in Figs. 14-16, catalytically active 1049HH ribozyme mediates a decrease in the expression of stromelysin expression in rabbit knees. The inhibition appears to be sequence-specific and ranges from 50-70%.

Example 14: Phosphorothioate-substituted Ribozymes inhibit stromelysin expression in Rabbit Knee

Ribozymes containing four phosphorothioate linkages at the 5' termini enhance ribozyme efficacy in mammalian cells. Referring to Fig. 17, applicant has designed and synthesized hammerhead ribozymes targeted to site 1049 within stromelysin RNA, wherein, the ribozymes contain five phosphorothioate linkages at their 5' and 3' termini. Additionally, these ribozymes contain 2'-O-methyl substitutions at 30 nucleotide positions, 2'-C-allyl substitution at U4 position and 2'-OH at five positions (Fig 17A). As described above, these ribozymes were administered to rabbit knees to test for ribozyme efficacy. The 1049 U4-C-allyl P=S active ribozyme shows greater than 50 % reduction in the level of stromelysin RNA in rabbit knee. Catalytically inactive version of the 1049 U4-C-allyl P=S ribozyme shows ~30% reduction in the level of stromelysin RNA.

Referring to Fig. 18, applicant has also designed and synthesized hammerhead ribozymes targeted to three distinct sites within stromelysin RNA, wherein, the ribozymes contain four phosphorothioate linkages at their 5' termini. Additionally, these ribozymes contain 2'-O-methyl substitutions at 29
5 nucleotide positions, 2'-amino substitutions at U4 and U7 positions and 2'-OH at five positions. As described above, these ribozymes were administered to rabbit knees to test for ribozyme efficacy. As shown in Figures 18-21, ribozymes targeted to sites 1049, 1363 and 1366 are all efficacious in rabbit knee. All three ribozymes decreased the level of stromelysin RNA in rabbit
10 knee by about 50 %.

Sequences and chemical modifications described in figures 17 and 18 are meant to be non-limiting examples. Those skilled in the art will recognize that similar embodiments with other ribozymes and ribozymes containing other chemical modifications can be readily generated using techniques
15 known in the art and are within the scope of the present invention.

Applicant has shown that chemical modifications, such as 6-methyl U and abasic (nucleotide containing no base) moieties can be substituted at certain positions within the ribozyme, for example U4 and U7 positions, without significantly effecting the catalytic activity of the ribozyme. Similarly,
20 3'-3' linked abasic inverted ribose moieties can be used to protect the 3' ends of ribozymes in place of an inverted T without effecting the activity of the ribozyme.

B7-1, B7-2, B7-3 and CD40 are attractive ribozyme targets by several criteria. The molecular mechanism of T cell activation is well-established.
25 Efficacy can be tested in well-defined and predictive animal models. The clinical end-point of graft rejection is clear. Since delivery would be *ex vivo*, treatment of the correct cell population would be assured. Finally, the disease condition is serious and current therapies are inadequate. Whereas protein-based therapies would induce anergy against all antigens encountered during
30 the several week treatment period, *ex vivo* ribozyme therapy provides a direct and elegant approach to truly donor-specific anergy.

Similarly, autoimmune diseases and allergies can be prevented or treated by reversing the devastating course of immune response to self-antigens. Specifically, nucleic acids of this inventions can dampen the response to naturally occurring antigens.

5 Example 15: B7-1, B7-2, B7-3 and/or CD40 Hammerhead ribozymes

By engineering ribozyme motifs we have designed several ribozymes directed against B7-1, B7-2, B7-3 and/or CD40 encoded mRNA sequences. These ribozymes were synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave target sequences *in*
10 *vitro* was evaluated.

Several common human cell lines are available that can be induced to express endogenous B7-1, B7-2, B7-3 and/or CD40 . Alternatively, murine splenic cells can be isolated and induced, to express B7-1 or B7-2, with IL-4 or recombinant CD40 ligand. B7-1 and B7-2 can be detected easily with
15 monoclonal antibodies. Use of appropriate fluorescent reagents and fluorescence-activated cell-sorting (FACS) will permit direct quantitation of surface B7-1 and B7-2 on a cell-by-cell basis. Active ribozymes are expected to directly reduce B7-1 or B7-2 expression. Ribozymes targeted to CD40 would prevent induction of B7-2 by CD40 ligand.

20 Several animal models of transplantation are available – Mouse, rat, Porcine model (Fodor et al., 1994, *Proc. Natl. Acad. Sci. USA* 91, 11153); or Baboon (reviewed by Nowak, 1994 *Science* 266, 1148). B7-1, B7-2, B7-3 and/or CD40 protein levels can be measured clinically or experimentally by FACS analysis. B7-1, B7-2, B7-3 and/or CD40 encoded mRNA levels will be
25 assessed by Northern analysis, RNase-protection, primer extension analysis and/or quantitative RT-PCR. Ribozymes that block the induction of B7-1, B7-2, B7-3 and/or CD40 activity and/or B7-1, B7-2, B7-3 and/or CD40 protein encoding mRNAs by more than 20% *in vitro* will be identified.

30 Several animals models of autoimmune disorders are available– allergic encephalomyelitis (EAE) in Lewis rats (Carlson et al., 1993 *Ann. N.Y. Acad. Sci.* 685, 86); animal models of multiple sclerosis (Wekerle et al., 1994 *Ann.*

Neurol. 36, s47) and rheumatoid arthritis (van Laar et al., 1994 Chem. Immunol. 58, 206).

Several animal models of allergy are available and are reviewed by Kemeny and Diaz-Sanchez, 1990, Clin. Exp. Immunol. 82, 423 and Pretolani
5 et al., 1994 Ann. N.Y.Acad. Sci. 725, 247).

RNA ribozymes and/or genes encoding them will be delivered by either free delivery, liposome delivery, cationic lipid delivery, adeno-associated virus vector delivery, adenovirus vector delivery, retrovirus vector delivery or plasmid vector delivery in these animal model experiments (see above). One
10 dose of a ribozyme vector that constitutively expresses the ribozyme or one or more doses of a stable anti-B7-1, B7-2, B7-3 and/or CD40 ribozymes or a transiently expressing ribozyme vector to donor APC, followed by infusion into the recipient may reduce the incidence of graft rejection. Alternatively, graft tissues may be treated as described above prior to transplantation.

15 Example 16: Synthesis of 6-methyl-uridine phosphoramidite

Referring to Figure 30, the suspension of 6-methyl-uracil (2.77g, 21.96 mmol) in the mixture of hexamethyldisilazane (50mL) and dry pyridine (50mL) was refluxed for three hours. The resulting clear solution of trimethylsilyl derivative of 6-methyl uracyl was evaporated to dryness and coevaporated 2
20 times with dry toluene to remove traces of pyridine. To the solution of the resulting clear oil, in dry acetonitrile, 1-O-acetyl-2',3',5'-tri-O-benzoyl-b-D-ribose (10.1g, 20 mmol) was added and the reaction mixture was cooled to 0°C. To the above stirred solution, trimethylsilyl trifluoromethanesulfonate (4.35 mL, 24 mmol) was added dropwise and the reaction mixture was stirred
25 for 1.5 h at 0°C and then 1h at room temperature. After that the reaction mixture was diluted with dichloromethane washed with saturated sodium bicarbonate and brine. The organic layer was evaporated and the residue was purified by flash chromatography on silica gel with ethylacetate-hexane (2:1) mixture as an eluent to give 9.5g (83%) of the compound 2 and 0.8g of
30 the corresponding N¹,N³-bis-derivative.

To the cooled (-10°C) solution of the compound (4.2g, 7.36 mmol) in the mixture of pyridine (60 mL) and methanol (10 mL) ice-cooled 2M aqueous

solution of sodium hydroxide (16 mL) was added with constant stirring. The reaction mixture was stirred at -10°C for additional 30 minutes and then neutralized to pH 7 with Dowex 50 (Py⁺). The resin was filtered off and washed with a 200 mL mixture of H₂O - Pyridine (4:1). The combined "mother liquor" and the washings were evaporated to dryness and dried by multiple coevaporation with dry pyridine. The residue was redissolved in dry pyridine and then mixed with dimethoxytrityl chloride (2.99g, 8.03 mmol). The reaction mixture was left overnight at room temperature. Reaction was quenched with methanol (25 mL) and the mixture was evaporated. The residue was dissolved in dichloromethane, washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by flash chromatography on silica gel using linear gradient of MeOH (2% to 5%) in CH₂Cl₂ as eluent to give 3.4g (83%) of the compound 6.

15 Example 17: Synthesis of 6-methyl-cytidine phosphoramidite

Triethylamine (13.4 ml, 100 mmol) was added dropwise to a stirred ice-cooled mixture of 1,2,4-triazole (6.22g, 90 mmol) and phosphorous oxychloride (1.89 ml, 20 mmol) in 50 ml of anhydrous acetonitrile. To the resulting suspension the solution of 2',3',5'-tri-O-Benzoyl-6-methyl uridine (5.7g, 10 mmol) in 30 ml of acetonitrile was added dropwise and the reaction mixture was stirred for 4 hours at room temperature. Then it was concentrated in vacuo to minimal volume (not to dryness). The residue was dissolved in chloroform and washed with water, saturated aq sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and the solvent was removed in vacuo. The residue was dissolved in 100 ml of 1,4-dioxane and treated with 50 mL of 29% aq NH₄OH overnight. The solvents were removed in vacuo. The residue was dissolved in the mixture of pyridine (60 mL) and methanol (10 mL), cooled to -15°C and ice-cooled 2M aq solution of sodium hydroxide was added under stirring. The reaction mixture was stirred at -10 to -15°C for additional 30 minutes and then neutralized to pH 7 with Dowex 50 (Py⁺). The resin was filtered off and washed with 200 mL of the mixture H₂O - Py (4:1). The combined mother liquor and washings were evaporated to dryness. The residue was crystallized from aq methanol to give 1.6g (62%) of 6-methyl cytidine.

To the solution of 6-methyl cytidine (1.4g, 5.44 mmol) in dry pyridine 3.11 mL of trimethylchlorosilane was added and the reaction mixture was stirred for 2 hours at room temperature. Then acetic anhydride (0.51 mL, 5.44 mmol) was added and the reaction mixture was stirred for additional 3 hours at room temperature. TLC showed disappearance of the starting material and the reaction was quenched with MeOH (20 mL), ice-cooled and treated with water (20 mL, 1 hour). The solvents were removed in vacuo and the residue was dried by four coevaporations with dry pyridine. Finally it was redissolved in dry pyridine and dimethoxytrityl chloride (2.2 g, 6.52 mmol) was added. The reaction mixture was stirred overnight at room temperature and quenched with MeOH (20 mL). The solvents were removed in vacuo. The remaining oil was dissolved in methylene chloride, washed with saturated sodium bicarbonate and brine. The organic layer was separated and evaporated and the residue was purified by flash chromatography on silica gel with the gradient of MeOH in methylene chloride (3% to 5%) to give 2.4 g (74%) of the compound (4).

Example 18: Synthesis of 6-aza-uridine and 6-aza-cytidine

To the solution of 6-aza uridine (5g, 20.39 mmol) in dry pyridine dimethoxytrityl chloride (8.29g, 24.47 mmol) was added and the reaction mixture was left overnight at room temperature. Then it was quenched with methanol (50 mL) and the solvents were removed in vacuo. The remaining oil was dissolved in methylene chloride and washed with saturated aq sodium bicarbonate and brine. The organic layer was separated and evaporated to dryness. The residue was additionally dried by multiple coevaporations with dry pyridine and finally dissolved in dry pyridine. Acetic anhydride (4.43 mL, 46.7 mmol) was added to the above solution and the reaction mixture was left for 3 hours at room temperature. Then it was quenched with methanol and worked-up as above. The residue was purified by flash chromatography on silica gel using mixture of 2% of MeOH in methylene chloride as an eluent to give 9.6g (75%) of the compound.

Triethylamine (23.7 ml, 170.4 mmol) was added dropwise to a stirred ice-cooled mixture of 1,2,4-triazole (10.6g, 153.36 mmol) and phosphorous oxychloride (3.22 ml, 34.08 mmol) in 100 ml of anhydrous acetonitrile. To the resulting suspension the solution of 2',3'-di-O-Acetyl-5'-O-Dimethoxytrityl-6-

aza Uridine (7.13g, 11.36 mmol) in 40 ml of acetonitrile was added dropwise and the reaction mixture was stirred for 6 hours at room temperature. Then it was concentrated in vacuo to minimal volume (not to dryness). The residue was dissolved in chloroform and washed with water, saturated aq sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and the solvent was removed in vacuo. The residue was dissolved in 150 ml of 1,4-dioxane and treated with 50 mL of 29% aq NH₄OH for 20 hours at room temperature. The solvents were removed in vacuo. The residue was purified by flash chromatography on silica gel using linear gradient of MeOH (4% to 10%) in methylene chloride as an eluent to give 3.1g (50%) of azacytidine.

To the stirred solution of 5'-O-Dimethoxytrityl-6-aza cytidine (3g, 5.53 mmol) in anhydrous pyridine trimethylchloro silane (2.41 mL, 19 mmol) was added and the reaction mixture was left for 4 hours at room temperature. Then acetic anhydride (0.63 mL, 6.64 mmol) was added and the reaction mixture was stirred for additional 3 hours at room temperature. After that it was quenched with MeOH (15 mL) and the solvents were removed in vacuo. The residue was treated with 1M solution of tetrabutylammonium fluoride in THF (20°, 30 min) and evaporated to dryness.. The remaining oil was dissolved in methylene chloride, washed with saturated aq sodium bicarbonate and water. The separated organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by flash chromatography on silica gel using 4% MeOH in methylene chloride as an eluent to give 2.9g (89.8%) of the compound.

General Procedure for the Introducing of the TBDMS-Group: To the stirred solution of the protected nucleoside in 50 mL of dry THF and pyridine (4 eq) AgNO₃ (2.4 eq) was added. After 10 minutes tert-butyldimethylsilyl chloride (1.5 eq) was added and the reaction mixture was stirred at room temperature for 12 hours. The resulted suspension was filtered into 100 mL of 5% aq NaHCO₃. The solution was extracted with dichloromethane (2x100 mL). The combined organic layer was washed with brine, dried over Na₂SO₄ and evaporated. The residue was purified by flash chromatography on silica gel with hexanes-ethylacetate (3:2) mixture as eluent.

General Procedure for Phosphitylation: To the ice-cooled stirred solution of protected nucleoside (1 mmol) in dry dichloromethane (20 mL) under argon blanket was added dropwise via syringe the premixed solution of N,N-diisopropylethylamine (2.5eq) and 2-cyanoethyl N'N-diisopropylchlorophosphoramidite (1.2 eq) in dichloromethane (3 mL). Simultaneously via another syringe N-methylimidazole (1 eq) was added and stirring was continued for 2 hours at room temperature. After that the reaction mixture was again ice-cooled and quenched with 15 ml of dry methanol. After 5 min stirring, the mixture was concentrated in vacuo (<40°C) and purified by flash chromatography on silica gel using hexanes-ethylacetate mixture contained 1% triethylamine as an eluent to give corresponding phosphoroamidite as white foam.

Example 19: RNA cleavage activity of HHA ribozyme substituted with 6-methyl-Uridine

Hammerhead ribozymes targeted to site A (see Fig. 31) were synthesized using solid-phase synthesis, as described above. U4 position was modified with 6-methyl-uridine.

RNA cleavage assay *in vitro*:

Substrate RNA is 5' end-labeled using [γ - 32 P] ATP and T4 polynucleotide kinase (US Biochemicals). Cleavage reactions were carried out under ribozyme "excess" conditions. Trace amount (≤ 1 nM) of 5' end-labeled substrate and 40 nM unlabeled ribozyme are denatured and renatured separately by heating to 90°C for 2 min and snap-cooling on ice for 10 -15 min. The ribozyme and substrate are incubated, separately, at 37°C for 10 min in a buffer containing 50 mM Tris-HCl and 10 mM MgCl₂. The reaction is initiated by mixing the ribozyme and substrate solutions and incubating at 37°C. Aliquots of 5 μ l are taken at regular intervals of time and the reaction is quenched by mixing with equal volume of 2X formamide stop mix. The samples are resolved on 20 % denaturing polyacrylamide gels. The results are quantified and percentage of target RNA cleaved is plotted as a function of time.

Referring to Fig. 32, hammerhead ribozymes containing 6-methyl-uridine modification at U4 position cleave the target RNA efficiently.

Example 20: RNA cleavage activity of HHB ribozyme substituted with 6-methyl-Uridine

Hammerhead ribozymes targeted to site B (see Fig. 33) were synthesized using solid-phase synthesis, as described above. U4 and U7 positions were
5 modified with 6-methyl-uridine.

RNA cleavage reactions were carried out as described above. Referring to Fig. 34, hammerhead ribozymes containing 6-methyl-uridine modification at U4 and U7 positions cleave the target RNA efficiently.

Example 21: RNA cleavage activity of HHC ribozyme substituted with 6-methyl-Uridine

Hammerhead ribozymes targeted to site C (see Fig. 35) were synthesized using solid-phase synthesis, as described above. U4 and U7 positions were
10 modified with 6-methyl-uridine.

RNA cleavage reactions were carried out as described above. Referring to
15 Fig. 36, hammerhead ribozymes containing 6-methyl-uridine modification at U4 positions cleave the target RNA efficiently.

Sequences listed in Figure 23, 31, 33, 35, and others and the modifications described in these figures are meant to be non-limiting examples. Those skilled in the art will recognize that variants (base-substitutions, deletions, insertions,
20 mutations, chemical modifications) of the ribozyme and RNA containing other 2'-hydroxyl group modifications, including but not limited to amino acids, peptides and cholesterol, can be readily generated using techniques known in the art, and are within the scope of the present invention.

Example 22: Inhibition of Rat smooth muscle cell proliferation by 6-methyl-U substituted ribozyme HHA.

Hammerhead ribozyme (HHA) is targeted to a unique site (site A) within c-myc mRNA. Expression of c-myc protein has been shown to be essential for the proliferation of rat smooth muscle cell (Brown *et al.*, 1992 *J. Biol. Chem.* 267, 4625).
25

The ribozymes that cleaved site A within *c-myb* RNA described above were assayed for their effect on smooth muscle cell proliferation. Rat vascular smooth muscle cells were isolated and cultured as described (Stinchcomb *et al.*, *supra*). HHA ribozymes were complexed with lipids and delivered into rat smooth muscle cells. Serum-starved cells were stimulated as described by Stinchcomb *et al.*, *supra*. Briefly, serum-starved smooth muscle cells were washed twice with PBS, and the RNA/lipid complex was added. The plates were incubated for 4 hours at 37°C. The medium was then removed and DMEM containing 10% FBS, additives and 10 µM bromodeoxyuridine (BrdU) was added. In some wells, FBS was omitted to determine the baseline of unstimulated proliferation. The plates were incubated at 37°C for 20-24 hours, fixed with 0.3% H₂O₂ in 100% methanol, and stained for BrdU incorporation by standard methods. In this procedure, cells that have proliferated and incorporated BrdU stain brown; non-proliferating cells are counter-stained a light purple. Both BrdU positive and BrdU negative cells were counted under the microscope. 300-600 total cells per well were counted. In the following experiments, the percentage of the total cells that have incorporated BrdU (% cell proliferation) is presented. Errors represent the range of duplicate wells. Percent inhibition then is calculated from the % cell proliferation values as follows: % inhibition = 100 - 100 (Ribozyme - 0% serum)/(Control - 0% serum).

Referring to Figure 37, active ribozymes substituted with 6-methyl-U at position 4 of HHA were successful in inhibiting rat smooth muscle cell proliferation. A catalytically inactive ribozyme (inactive HHA), which has two base substitutions within the core (these mutations inactivate a hammerhead ribozyme; Stinchcomb *et al.*, *supra*), does not significantly inhibit rat smooth muscle cell proliferation.

Example 23: Inhibition of stromelysin production in human synovial fibroblast cells by 6-methyl-U substituted ribozyme HHC.

Hammerhead ribozyme (HHC) is targeted to a unique site (site C) within *stromelysin* mRNA.

The general assay was as described (Draper *et al.*, *supra*). Briefly, fibroblasts, which produce stromelysin, are serum-starved overnight and ribozymes or controls are offered to the cells the next day. Cells were maintained in serum-free media. The ribozyme were applied to the cells as free ribozyme, or in association with various delivery vehicles such as cationic

lipids (including Transfectam™, Lipofectin™ and Lipofectamine™), conventional liposomes, non-phospholipid liposomes or biodegradable polymers. At the time of ribozyme addition, or up to 3 hours later, Interleukin-1 α (typically 20 units/ml) can be added to the cells to induce a large increase in stromelysin expression. The production of stromelysin can then be monitored over a time course, usually up to 24 hours.

Supernatants were harvested 16 hours after IL-1 induction and assayed for stromelysin expression by ELISA. Polyclonal antibody against Matrix Metalloproteinase 3 (Biogenesis, NH) was used as the detecting antibody and anti-stromelysin monoclonal antibody was used as the capturing antibody in the sandwich ELISA (Maniatis *et al.*, *supra*) to measure stromelysin expression.

Referring to Figure 38, HHC ribozyme containing 6-methyl-U modification, caused a significant reduction in the level of stromelysin protein production. Catalytically inactive HHC had no significant effect on the protein level.

Example 24: Synthesis of pyridin-2(4)-one nucleoside 3'-phosphoramidites

General procedure for the preparation of 1-(2,3,5-tri-O-benzoyl- β -D-ribofuranosyl)-2(4)-pyridones (3) and (9)

Referring to Figure 39, 2- or 4-hydroxypyridine (1) or (8) (2.09 g, 22 mmol), 1-O-acetyl-2,3,5-tri-O-benzoyl- β -D-ribofuranose (2) (10.08 g, 20 mmol) and BSA (5.5 ml, 22 mmol) were dissolved in dry acetonitrile (100 ml) under argon at 70°C (oil bath) and the mixture stirred for 10 min. Trimethylsilyl trifluoromethanesulfonate (TMSTf) (5.5 ml, 28.5 mmol) was added and the mixture was stirred for an additional hour for 1 or four hours for 8. The mixture was then cooled to room temperature (RT) followed by dilution, with CHCl₃ (200 ml), and extraction, with sat. aq. NaHCO₃ solution. The organic layer was washed with brine, dried (Na₂SO₄) and evaporated to dryness *in vacuo*. The residue was chromatographed on the column of silica gel; 1-5% gradient of methanol in dichloromethane was used for purification of 3 (98% yield) and 2-10% gradient of methanol in dichloromethane for purification of 9 (84% yield).

1-(β -D-Ribofuranosyl)-2(4)-pyridones (4) and (10)

3 or 9 (18 mmol) was dissolved in 0.3M NaOCH₃ (150 ml) and the solution was stirred at RT for 1 hour. The mixture was then neutralized, with Dowex 50WX8 (Py⁺), the ion-exchanger was filtered off and the filtrate was concentrated to a syrup *in vacuo*. The residue was dissolved in water (100 ml) and the solution was washed with chloroform (2 x 50 ml) and ether (2 x 50 ml). The aqueous layer was evaporated to dryness and the residue was then crystallized from ethyl acetate (3.9 g, 91% 4; Niedballa *et al.*, *Nucleic Acid Chemistry*, Part 1, Townsend, L.B. and Tipson, R.S., Ed.; J. Wiley & Sons, Inc.; New York, 1978, p 481-484); 10 (Niedballa and Vorbrüggen, *J. Org. Chem.* 1974, 39, 3668-3671) was crystallized from ethanol (3.6 g, 84%).

1-(2-O-TBDMSi-5-O-DMT- β -D-ribofuranosyl)-2(4)-pyridones

4 or 10 was 5'-O-dimethoxytritylated according to the standard procedure (see Oligonucleotide Synthesis: A Practical Approach, M.J. Gait Ed.; IRL Press, Oxford, 1984, p 27) to yield 5 in 76% yield and pyridin-4-one derivative in 67% yield in the form of yellowish foams after silica gel column chromatography (0.5-10% gradient of methanol in dichloromethane). These compounds were treated with *t*-butyldimethylsilyl chloride under the conditions described by Hakimelahi *et al.*, *Can. J. Chem.* 1982, 60, 1106-1113, and the reaction mixtures were purified by the silica gel column chromatography (20-50% gradient of ethyl acetate in hexanes) to enable faster moving 2'-O-TBDMSi isomers (68.5% and 55%, respectively) as colorless foams.

1-[2-O-*t*-Butyldimethylsilyl-5-O-dimethoxytrityl-3-O-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)]-2(4)-pyridones (7) and (11)

1-(2-O-TBDMS-5-O-DMT- β -D-ribofuranosyl)-2(4)-pyridones were phosphitylated under conditions described by Tuschl *et al.*, *Biochemistry* 1993, 32, 11658-11668, and the products were isolated by silica gel column chromatography using 15-50% gradient of ethyl acetate in hexanes (1% Et₃N) for 7 (89% yield) and dichloromethane (1% Et₃N) for 11 (94% yield).

Phosphoramidites 7 and 11 were incorporated into ribozymes and substrates using the method of synthesis, deprotection, purification and testing

previously described (Wincott *et al.*, 1995 *supra*). The average stepwise coupling yields were ~98 %.

Example 25: Synthesis of 2-O-*t*-Butyldimethylsilyl-5-O-dimethoxytrityl-3-O-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-deoxy-1-phenyl- β -D-

5 ribofuranose (8) phosphoramidites

5-O-*t*-Butyldiphenylsilyl-2,3-O-isopropylidene-1-deoxy-1-phenyl- β -D-ribofuranose (3)

Referring to Figure 40, compound 3 was prepared using the procedure analogous to that described by Czerniecki and Ville, *J. Org. Chem.* 1989, 54, 610-612. Contrary to their result, we succeeded in obtaining the title compound, by using the more acid resistant *t*-butyldiphenylsilyl group for 5-O-protection, instead of *t*-butyldimethylsilyl.

1-Deoxy-1-phenyl- β -D-ribofuranose (5)

Compound 3 (1 g, 2.05 mmol) was dissolved in THF (20 ml) and the solution was mixed with 1M TBAF in THF (3 ml, 3 mmol). The reaction mixture was stirred at RT for 30 min followed by evaporation into a syrup. The residue was applied on to a silica gel column and eluted with hexanes followed by 5-70% gradient of ethyl acetate in hexanes. The 5-O-desilylated product was obtained as a colorless foam (0.62 g, 88% yield). This material was dissolved in 70% acetic acid and heated at 100°C (oil bath) for 30 min. Evaporation to dryness under reduced pressure and crystallization of the residual syrup from toluene resulted in 5 (0.49 g, 94% yield), mp 120-121°C.

2-O-*t*-Butyldimethylsilyl-5-O-dimethoxytrityl-1-deoxy-1-phenyl- β -D-ribofuranose (7)

Compound 5 (770 mg, 3.66 mmol) was 5-O-dimethoxytritylated according to the standard procedure (Oligonucleotide Synthesis: A Practical Approach, M.J. Gait Ed.; IRL Press, Oxford, 1984, p 27) to yield 1.4 g (75% yield) of 5-O-dimethoxytrityl derivative as a yellowish foam, following silica gel column chromatography (0.5-2% gradient of methanol in dichloromethane). This material was treated with *t*-butyldimethylsilyl chloride under the conditions described by Hakimelahi *et al.*, *Can. J. Chem.* 1982, 60, 1106-1113. and the reaction mixture

was purified by silica gel column chromatography (2-10% gradient of ethyl acetate in hexanes) to afford a slower moving 2'-O-TBDMSi isomer 7 (0.6 g, 35% yield) as a colorless foam. The faster migrating 3'-O-TBDMSi isomer 6 was also isolated (0.55 g, 32% yield).

5 2-O-*t*-Butyldimethylsilyl-5-O-dimethoxytrityl-3-O-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-deoxy-1-phenyl- β -D-ribofuranose (8)

Compound 7 (0.87 g, 1.39 mmol) was phosphitylated under conditions described by Tuschl *et al.*, *supra* and the product was isolated by silica gel column chromatography using 0.5% ethyl acetate in toluene (1% Et₃N) for elution
10 (0.85 g, 74% yield).

Example 26: Synthesis of pseudouridine, 3-methyluridine and 2,4,6-trimethoxy benzene nucleoside phosphoramidites

Starting with a pseudo uridine, 3-methyluridine or 2,4,6-trimethoxy benzene nucleoside (Gasparutto *et al.*, *Nucleic Acid Res.* 1992 20, 5159-5166; Kalvoda
15 and Farkas, *Nucleic Acid Chemistry*, Part 1, Townsend, L.B. and Tipson, R.S., Ed.: J. Wiley & Sons, Inc.; New York, 1978, p 481-484), phosphoramidites can be prepared by standard protocols described below (Figure 41).

General Procedure for the Introducing of the TBDMS-Group: To the stirred solution of the protected nucleoside in 50 mL of dry THF and pyridine (4 eq)
20 AgNO₃ (2.4 eq) was added. After 10 minutes tert-butyldimethylsilyl chloride (1.5 eq) was added and the reaction mixture was stirred at room temperature for 12 hours. The resulted suspension was filtered into 100 mL of 5% aq NaHCO₃. The solution was extracted with dichloromethane (2x100 mL). The combined organic layer was washed with brine, dried over Na₂SO₄ and evaporated. The residue
25 was purified by flash chromatography on silica gel with hexanes-ethylacetate (3:2) mixture as eluent.

General Procedure for Phosphitylation: To the ice-cooled stirred solution of protected nucleoside (1 mmol) in dry dichloromethane (20 mL) under argon blanket was added dropwise via syringe the premixed solution of *N,N*-
30 diisopropylethylamine (2.5eq) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (1.2 eq) in dichloromethane (3 mL).

Simultaneously via another syringe N-methylimidazole (1 eq) was added and stirring was continued for 2 hours at room temperature. After that the reaction mixture was again ice-cooled and quenched with 15 ml of dry methanol. After 5 min stirring, the mixture was concentrated in vacuo (<40°C) and purified by flash chromatography on silica gel using hexanes-ethylacetate mixture contained 1% triethylamine as an eluent to give corresponding phosphoroamidite as white foam.

Pseudouridine, 3-methyluridine or 2,4,6-trimethoxy benzene phosphoramidites were incorporated into ribozymes using solid phase synthesis as described by Wincott *et al.*, 1995 *supra*. The ribozymes were deprotected using the standard protocol described above with the exception of ribozymes with pseudouridine. Pseudouridine-modified ribozymes were deprotected first by incubation at room temperature, instead of at 55°C, for 24 hours in a mixture of ethanolic ammonia (3:1).

15 Example 27: Synthesis of dihydrouridine phosphoramidites

Referring to Figure 42, dihydrouridine phosphoramidite was synthesized based on the method described in Chaix *et al.*, 1989 *Nucleic Acid Res.* 17, 7381-7393 with certain improvements:

- 20 i. Uridine (1; 10g, 41mmoles) was dissolved in 200 ml distilled water and to the solution 2g of Rh (10% on alumina) was added. The slurry was brought to 60 psi of hydrogen, and hydrogenation was continued for 16hrs. Reaction was monitored by disappearance of UV absorbing material. All of starting material was converted to dihydrouridine (DHU) and tetrahydrouridine (2:1 based on NMR). Tetrahydrouridine was not removed at this step.
- 25 ii. Dihydrouridine (2; 10g, 41mmoles) was dissolved in 400ml dry pyridine; dimethylaminopyridine (0.244g, 2mmoles), triethylamine (7.93ml, 56mmoles), and dimethoxytritylchloride (16.3g, 48mmoles) were added and stirred under argon overnight. The reaction was quenched with 50ml methanol, extracted with 400ml 5% sodium bicarbonate, and then 400ml brine. The organic phase was dried over sodium sulphate, filtered, and then dried to a foam. 5'-DMT-DHU (3) was purified by silica gel chromatography (dichloromethane with 0.5-5% gradient of methanol; final yield = 9g; 16.4mmoles).
- 30

III. 5'-DMT-DHU (3; 9.0g, 16.4mmoles) was dissolved in 150ml dry THF. Pyridine (4.9ml, 60mmoles) and silver nitrate (3.35g, 19.7mmoles) were added at room temperature and stirred under argon for 10min., then *tert*-butyldimethylsilylchloride (tBDMS-Cl; 3.0g, 19.7mmoles) was added and the slurry was stirred under argon overnight. The reaction was filtered over celite into 500ml aqueous 5% sodium bicarbonate and then extracted with 200ml chloroform. The organic phase was washed with 250ml brine, dried over sodium sulfate, and then evaporated to a yellow foam. 2'-tBDMS, 5'-DMT-DHU (5) was purified by silica gel chromatography away from the 3'-tBDMS, 5'-DMT-DHU (4) (hexanes with 10-50% gradient ether; final yield = 5.1g; 7.7mmoles), dried over sodium sulfate, filtered, and then dried to a white powder. The product was kept under high vacuum for 48hrs.

iv. 5'-DMT, 2'-tBDMS-DHU (5; 2.10g, 3.17mmoles) was dissolved in 40ml anhydrous dichloromethane. NN-dimethylaminopyridine (2.21ml, 12.7mmoles), N-methylimidazole (1.27ml, 1.59mmoles), and chloro-diisopropylcyanoethylphosphoramidite (1.2ml, 5.22mmoles) were added and the reaction was stirred under argon for 3hrs. The reaction was quenched with 4ml anhydrous methanol and then evaporated to an oil. Final product (6) was purified by silica gel chromatography (dichloromethane with 0-1% ethanol; 1% triethylamine; final yield = 2.2g; 2.5mmoles).

The dihydrouridine was incorporated into ribozymes using solid phase synthesis as described by Wincott *et al.*, 1995 *supra*. with improvements—nucleoside-oxalyl-polystyrene derivatized support (Alul *et. al.* Nucleic Acids Res., 1991, 19, 1527-1532) was used. The ribozyme containing the dihydrouridine substitution was deprotected using 30% methyl amine in anhydrous ethanol for 15 min. at room temperature and subsequent treatment with *tert*-butyl-ammonium fluoride in anhydrous THF for 24 hrs. at room temperature.

Example 28: Synthesis of 2-O-*t*-Butyldimethylsilyl-5-O-dimethoxytrityl-3-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)-1-deoxy-1-naphthyl-β-D-ribofuranose (7) phosphoramidites

1-Deoxy-1-naphthyl-β-D-ribofuranose (4)

Referring to Figure 45, the title compound was synthesized from naphthalene 1 and tetra-*O*-acetyl- β -D-ribofuranose 2 according to the procedure of Ohruj *et al.* *Agr. Biol. Chem.* 1972, 36, 1651-1653.

5 2-*O*-*t*-Butyldimethylsilyl-5-*O*-dimethoxytrityl-3-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-deoxy-1-naphthyl- β -D-ribofuranose (7)

7 was synthesized in three steps from 4: a) 5'-*O*-dimethoxytritylation using 4,4'-dimethoxytrityl triflate, followed by chromatographic separation of α and β anomer, respectively; b) 2'-*O*-silylation was carried out as described by Hakimelahi *et al.*, 1982 *supra* (32% yield); c) 3'-*O*-phosphitylation was carried out essentially as described by Tuschl *et al.*, 1993 *supra* (85% yield).

This phosphoramidite is incorporated into ribozymes using solid phase synthesis as described by Wincott *et al.*, 1995 *supra*. The ribozyme containing naphthyl substitution was deprotected using the standard protocol described above.

15 Example 29: Synthesis of 2-*O*-*t*-Butyldimethylsilyl-5-*O*-Dimethoxytrityl-3-*O*-(2-Cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-Deoxy-1-(*p*-Aminophenyl)- β -D-Ribofuranose phosphoramidites

5-*O*-*t*-Butyldiphenylsilyl-2,3-*O*-isopropylidene-1-deoxy-1-(*p*-bromophenyl)- β -D-ribofuranose (3)

20 Referring to Figure 46, 3 was prepared from 4-bromo-1-lithiobenzene and *t*-butyldiphenylsilyl-2,3-*O*-isopropylidene-D-ribo-1,4-lactone using the procedure analogous to that described by Czerniecki and Ville, *J. Org. Chem.* 1989, 54, 610-612. Contrary to their result, we succeeded in obtaining the title compound, by using instead of *t*-butyldimethylsilyl the more acid resistant *t*-butyldiphenylsilyl group for 5-*O*-protection.

25 5-*O*-*t*-Butyldiphenylsilyl-2,3-*O*-isopropylidene-1-deoxy-1-(*p*-aminophenyl)- β -D-ribofuranose (5)

Compound 3 was aminated using liquid ammonia and CuI as described by Piccirilli *et al.* *Helv. Chim. Acta* 1991, 74, 397-406 to give the title compound in 63% yield.

5-O-*t*-Butyldiphenylsilyl-2,3-O-isopropylidene-1-deoxy-1-[p-(N-TFA)aminophenyl]- β -D-ribofuranose (6)

5 (1.2 g, 2.88 mmol) in dry pyridine (20 ml) was treated with trifluoroacetic anhydride (0.5 ml, 3.6 mmol) for 1 hour at 0 °C. The reaction mixture was then quenched with methanol (5 ml) and evaporated to a syrup. The syrup was partitioned between 5% aq. NaHCO₃ and dichloromethane, organic layer was dried (Na₂SO₄) and evaporated to dryness under reduced pressure. This material was used without further purification in the next step.

1-Deoxy-1-[p-(N-TFA)aminophenyl]- β -D-ribofuranose (7)

10 The title compound was prepared from 6 in an identical manner as for the synthesis of deblocked phenyl analog; (82% overall yield for 5'-O-desilylation and the cleavage of 2',3'-O-isopropylidene group).

2-O-*t*-Butyldimethylsilyl-5-O-dimethoxytrityl-3-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)-1-deoxy-1-[p-(N-TFA)aminophenyl]- β -D-ribofuranose (10)

15 Using the same three step sequence as for the phenyl analog, 10 was prepared from 7 in 32% overall yield.

20 This phosphoramidite is incorporated into ribozymes using solid phase synthesis as described by Wincott *et al.*, 1995 *supra*. The ribozyme containing aminophenyl substitution was deprotected using the standard protocol described above.

Example 30: RNA cleavage reactions catalyzed by HH-B substituted with modified bases

25 Hammerhead ribozymes targeted to site B (see Fig. 43A) were synthesized using solid-phase synthesis, as described above. U4 and U7 positions were substituted with various base-modifications shown in Figure 43B.

30 RNA cleavage reactions were carried out as described above. Referring to Fig. 43B, hammerhead ribozymes containing base modifications at positions 4 or 7 cleave the target RNA to varying degrees of efficiency. Some of the base modifications at position 7 appear to enhance the catalytic efficiency of the

hammerhead ribozymes compared to a standard base at that position (see Figure 43B, pyridin-4-one, phenyl and 3-methyl U modifications).

5 HH-B ribozymes with either pyridin-4-one or phenyl substitution at position 7 were further characterized (Figure 44). It appears that HH-B ribozyme with pyridin-4-one modification at position 7 cleaves RNA with a 10 fold higher k_{cat} when compared to a ribozyme with a U at position 7 (compare Figure 44 A with 44 B). HH-B ribozyme with a phenyl group at position 7 cleaves RNA with a 3 fold higher k_{cat} when compared to a hammerhead ribozyme with U at position 7 (see Figure 44C).

10 Sequences listed in Figure 23, 31, 33, 35, 43 and the modifications described in these figures are meant to be non-limiting examples. Those skilled in the art will recognize that variants (base-substitutions, deletions, insertions, mutations, chemical modifications) of the ribozyme and RNA containing other 2'-hydroxyl group modifications, including but not limited to amino acids, peptides
15 and cholesterol, can be readily generated using techniques known in the art, and are within the scope of the present invention.

Example 31: 2'-deoxy-2'-alkylnucleotides

Table D2 is a summary of specified catalytic parameters (t_A and t_S) on short substrates *in vitro*, and stabilities of the noted modified catalytic nucleic
20 acids in human serum. U4 and U7 refer to the uracil bases noted in Figure 1. Modifications at the 2'-position are shown in the table.

Table D2

Entry	Modification	$t_{1/2}$ (m) Activity (t_A)	$t_{1/2}$ (m) Stability (t_S)	$\beta = t_S/t_A$ $\times 10$
1	U4 & U7 = U	1	0.1	1
2	U4 & U7 = 2'-O-Me-U	4	260	650
3	U4 = 2'=CH ₂ -U	6.5	120	180
4	U7 = 2'=CH ₂ -U	8	280	350
5	U4 & U7 = 2'=CH ₂ -U	9.5	120	130
6	U4 = 2'=CF ₂ -U	5	320	640
7	U7 = 2'=CF ₂ -U	4	220	550
8	U4 & U7 = 2'=CF ₂ -U	20	320	160
9	U4 = 2'-F-U	4	320	800
10	U7 = 2'-F-U	8	400	500
11	U4 & U7 = 2'-F-U	4	300	750
12	U4 = 2'-C-Allyl-U	3	>500	>1700
13	U7 = 2'-C-Allyl-U	3	220	730
14	U4 & U7 = 2'-C-Allyl-U	3	120	400
15	U4 = 2'-araF-U	5	>500	>1000
16	U7 = 2'-araF-U	4	350	875
17	U4 & U7 = 2'-araF-U	15	500	330
18	U4 = 2'-NH ₂ -U	10	500	500
19	U7 = 2'-NH ₂ -U	5	500	1000
20	U4 & U7 = 2'-NH ₂ -U	2	300	1500
21	U4 = dU	6	100	170
22	U4 & U7 = dU	4	240	600

Figure 47 shows base numbering of a hammerhead motif in which the numbering of various nucleotides in a hammerhead ribozyme is provided. Referring to Figure 47, the preferred sequence of a hammerhead ribozyme in a 5'- to 3'-direction of the catalytic core is CUGANGAG[base paired with]CGAAA. In this invention, the use of 2'-C-alkyl substituted nucleotides that maintain or enhance the catalytic activity and or nuclease resistance of the hammerhead ribozyme is described. Although substitutions of any nucleotide with any of the modified nucleotides shown in Figure 48 are possible, and were indeed synthesized, the basic structure composed of primarily 2'-O-Me nucleotides with selected substitutions was chosen to maintain maximal catalytic activity (Yang *et al. Biochemistry* 1992, 31, 5005-5009 and Paoletta *et al. EMBO J.* 1992, 11, 1913-1919) and ease of synthesis, but is not limiting to this invention.

Ribozymes from Figure 47 and Table D2 were synthesized and assayed for catalytic activity and nuclease resistance. With the exception of entries 8 and 17, all of the modified ribozymes retained at least 1/10 of the wild-type catalytic activity. From Table D2, all 2'-modified ribozymes showed very large and significant increases in stability in human serum (shown) and in the other fluids described below (Example 3, data not shown). The order of most aggressive nuclease activity was fetal bovine serum > human serum > human plasma > human synovial fluid. As an overall measure of the effect of these 2'-substitutions on stability and activity, a ratio β was calculated (Table D2). This β value indicated that all modified ribozymes tested had significant, >100 - >1700 fold, increases in overall stability and activity. These increases in β indicate that the lifetime of these modified ribozymes *in vivo* are significantly increased which should lead to a more pronounced biological effect.

More general substitutions of the 2'-modified nucleotides from Figure 48 also increased the $t_{1/2}$ of the resulting modified ribozymes. However the catalytic activity of these ribozymes was decreased > 10-fold.

In Figure 53 compound 37 may be used as a general intermediate to prepare derivatized 2'-C-alkyl phosphoramidites, where X is CH₃, or an alkyl, or other group described above.

The following are other non-limiting examples showing the synthesis of nucleic acids using 2'-C-alkyl substituted phosphoramidites, the syntheses of the amidites, their testing for enzymatic activity and nuclease resistance. These examples are diagrammed in Figs 48-54.

5 Example 32: Synthesis of Hammerhead Ribozymes Containing 2'-Deoxy-2'-Alkyl nucleotides & Other 2'-Modified Nucleotides

The method of synthesis used generally follows the procedure for normal RNA synthesis as described in Usman, N.; Ogilvie, K.K.; Jiang, M.-Y.; Cedergren, R.J. *J. Am. Chem. Soc.* **1987**, *109*, 7845-7854 and in
10 Scaringe, S.A.; Franklyn, C.; Usman, N. *Nucleic Acids Res.* **1990**, *18*, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end (compounds 10, 12, 17, 22, 31, 18, 26, 32, 36 and 38). Other 2'-modified phosphoramidites were prepared according to: 3 & 4, Eckstein *et al.*
15 *International Publication* No. WO 92/07065; and 5 Kois *et al.* *Nucleosides & Nucleotides* **1993**, *12*, 1093-1109. The average stepwise coupling yields were ~98%. The 2'-substituted phosphoramidites were incorporated into hammerhead ribozymes as shown in Figure 5. However, these 2'-alkyl substituted phosphoramidites may be incorporated not only into hammerhead
20 ribozymes, but also into hairpin, hepatitis delta virus, Group I or Group II intron catalytic nucleic acids, or into antisense oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 33: Ribozyme Activity Assay

Purified 5'-end labeled RNA substrates (15-25-mers) and purified 5'-end
25 labeled ribozymes (~36-mers) were both heated to 95 °C, quenched on ice and equilibrated at 37 °C, separately. Ribozyme stock solutions were 1 mM, 200 nM, 40 nM or 8 nM and the final substrate RNA concentrations were ~ 1 nM. Total reaction volumes were 50 µL. The assay buffer was 50 mM Tris-Cl, pH 7.5 and 10 mM MgCl₂. Reactions were initiated by mixing substrate and
30 ribozyme solutions at t = 0. Aliquots of 5 µL were removed at time points of 1, 5, 15, 30, 60 and 120 m. Each time point was quenched in formamide loading buffer and loaded onto a 15% denaturing polyacrylamide gel for analysis.

Quantitative analyses were performed using a phosphorimager (Molecular Dynamics).

Example 34: Stability Assay

500 pmol of gel-purified 5'-end-labeled ribozymes were precipitated in ethanol and pelleted by centrifugation. Each pellet was resuspended in 20 mL of appropriate fluid (human serum, human plasma, human synovial fluid or fetal bovine serum) by vortexing for 20 s at room temperature. The samples were placed into a 37 °C incubator and 2 mL aliquots were withdrawn after incubation for 0, 15, 30, 45, 60, 120, 240 and 480 m. Aliquots were added to 20 mL of a solution containing 95% formamide and 0.5X TBE (50 mM Tris, 50 mM borate, 1 mM EDTA) to quench further nuclease activity and the samples were frozen until loading onto gels. Ribozymes were size-fractionated by electrophoresis in 20% acrylamide/8M urea gels. The amount of intact ribozyme at each time point was quantified by scanning the bands with a phosphorimager (Molecular Dynamics) and the half-life of each ribozyme in the fluids was determined by plotting the percent intact ribozyme vs the time of incubation and extrapolation from that graph.

Example 35: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-O-Phenoxythio-carbonyl-Uridine (7)

To a stirred solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-uridine, 6, (15.1 g, 31 mmol, synthesized according to *Nucleic Acid Chemistry*, ed. Leroy Townsend, 1986 pp. 229-231) and dimethylaminopyridine (7.57 g, 62 mmol) a solution of phenylchlorothionoformate (5.15 mL, 37.2 mmol) in 50 mL of acetonitrile was added dropwise and the reaction stirred for 8 h. TLC (EtOAc:hexanes / 1:1) showed disappearance of the starting material. The reaction mixture was evaporated, the residue dissolved in chloroform, washed with water and brine, the organic layer was dried over sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography on silica gel with EtOAc:hexanes / 2:1 as eluent to give 16.44 g (85%) of 7.

Example 36: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-C-Allyl -Uridine (8)

To a refluxing, under argon, solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-2'-O-phenoxythiocarbonyl-uridine, **7**, (5 g, 8.03 mmol) and allyltributyltin (12.3 mL, 40.15 mmol) in dry toluene, benzoyl peroxide (0.5 g)
5 was added portionwise during 1 h. The resulting mixture was allowed to reflux under argon for an additional 7-8 h. The reaction was then evaporated and the product **8** purified by flash chromatography on silica gel with EtOAc:hexanes / 1:3 as eluent. Yield 2.82 g (68.7%).

Example 37: 5'-O-Dimethoxytrityl-2'-C-Allyl-Uridine (9)

10 A solution of **8** (1.25 g, 2.45 mmol) in 10 mL of dry tetrahydrofuran (THF) was treated with a 1 M solution of tetrabutylammoniumfluoride in THF (3.7 mL) for 10 m at room temperature. The resulting mixture was evaporated, the residue was loaded onto a silica gel column, washed with 1 L of chloroform, and the desired deprotected compound was eluted with chloroform:methanol /
15 9:1. Appropriate fractions were combined, solvents removed by evaporation, and the residue was dried by coevaporation with dry pyridine. The oily residue was redissolved in dry pyridine, dimethoxytritylchloride (1.2 eq) was added and the reaction mixture was left under anhydrous conditions overnight. The reaction was quenched with methanol (20 mL), evaporated,
20 dissolved in chloroform, washed with 5% aq. sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by flash chromatography on silica gel, EtOAc:hexanes / 1:1 as eluent, to give 0.85 g (57%) of **9** as a white foam.

Example 38: 5'-O-Dimethoxytrityl-2'-C-Allyl-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (10)

25 5'-O-Dimethoxytrityl-2'-C-allyl-uridine (0.64 g, 1.12 mmol) was dissolved in dry dichloromethane under dry argon. N,N-Diisopropylethylamine (0.39 mL, 2.24 mmol) was added and the solution was ice-cooled. 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (0.35 mL, 1.57 mmol) was added dropwise to
30 the stirred reaction solution and stirring was continued for 2 h at RT. The reaction mixture was then ice-cooled and quenched with 12 mL of dry methanol. After stirring for 5 m, the mixture was concentrated *in vacuo* (40 °C)

and purified by flash chromatography on silica gel using a gradient of 10-60% EtOAc in hexanes containing 1% triethylamine mixture as eluent. Yield: 0.78 g (90%), white foam.

5 Example 39: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-C-Allyl-N⁴-Acetyl-Cytidine (11)

Triethylamine (6.35 mL, 45.55 mmol) was added dropwise to a stirred ice-cooled mixture of 1,2,4-triazole (5.66 g, 81.99 mmol) and phosphorous oxychloride (0.86 mL, 9.11 mmol) in 50 mL of anhydrous acetonitrile. To the
10 resulting suspension a solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-2'-C-allyl uridine (2.32 g, 4.55 mmol) in 30 mL of acetonitrile was added dropwise and the reaction mixture was stirred for 4 h at room temperature. The reaction was concentrated *in vacuo* to a minimal volume (not to dryness). The residue was dissolved in chloroform and washed with water, saturated aq.
15 sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and the solvent was removed *in vacuo*. The resulting foam was dissolved in 50 mL of 1,4-dioxane and treated with 29% aq. NH₄OH overnight at room temperature. TLC (chloroform:methanol / 9:1) showed complete conversion of the starting material. The solution was evaporated, dried by
20 coevaporation with anhydrous pyridine and acetylated with acetic anhydride (0.52 mL, 5.46 mmol) in pyridine overnight. The reaction mixture was quenched with methanol, evaporated, the residue was dissolved in chloroform, washed with sodium bicarbonate and brine. The organic layer was dried over sodium sulfate, evaporated to dryness and purified by flash chromatography on silica gel (3% MeOH in chloroform). Yield 2.3 g (90%) as
25 a white foam.

Example 40: 5'-O-Dimethoxytrityl-2'-C-Allyl-N⁴-Acetyl-Cytidine

This compound was obtained analogously to the uridine derivative 9 in 55% yield.

Example 41: 5'-O-Dimethoxytrityl-2'-C-allyl-N⁴-Acetyl-Cytidine 3'-(2-Cyano-ethyl N,N-diisopropylphosphoramidite) (12)

2'-O-Dimethoxytrityl-2'-C-allyl-N⁴-acetyl cytidine (0.8 g, 1.31 mmol) was dissolved in dry dichloromethane under argon. N,N-Diisopropylethylamine
5 (0.46 mL, 2.62 mmol) was added and the solution was ice-cooled. 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (0.38 mL, 1.7 mmol) was added dropwise to a stirred reaction solution and stirring was continued for 2 h at room temperature. The reaction mixture was then ice-cooled and quenched with 12 mL of dry methanol. After stirring for 5 m, the mixture was
10 concentrated *in vacuo* (40 °C) and purified by flash chromatography on silica gel using chloroform:ethanol / 98:2 with 2% triethylamine mixture as eluent. Yield: 0.91 g (85%), white foam.

Example 42: 2'-Deoxy-2'-Methylene-Uridine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine
15 14 (Hansske,F.; Madej,D.; Robins,M. J. *Tetrahedron* 1984, 40, 125 and Matsuda,A.; Takenuki,K.; Tanaka,S.; Sasaki,T.; Ueda,T. *J. Med. Chem.* 19⁹¹, 34, 812) (2.2 g, 4.55 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column.
20 2'-Deoxy-2'-methylene-uridine (1.0 g, 3.3 mmol, 72.5%) was eluted with 20% MeOH in CH₂Cl₂.

Example 43: 5'-O-DMT-2'-Deoxy-2'-Methylene-Uridine (15)

2'-Deoxy-2'-methylene-uridine (0.91 g, 3.79 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl in pyridine (10 mL) was added
25 dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column
30 using EtOAc:hexanes as eluant to yield 15 (0.43 g, 0.79 mmol, 22%).

Example 44: 5'-O-DMT-2'-Deoxy-2'-Methylene-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (17)

1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl- β -D-ribofuranosyl)-uracil (0.43 g, 0.8 mmol) dissolved in dry CH_2Cl_2 (15 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.28 mL, 1.6 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.25 mL, 1.12 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product (0.3 g, 0.4 mmol, 50%) was purified by flash column chromatography over silica gel using a 25-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.42 (CH_2Cl_2 : MeOH / 15:1)

Example 45: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-Uridine

2'-Keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)uridine **14** (1.92 g, 12.6 mmol) and triphenylphosphine (2.5 g, 9.25 mmol) were dissolved in diglyme (20 mL), and heated to a bath temperature of 160 °C. A 60 °C solution of sodium chlorodifluoroacetate in diglyme (50 mL) was added (dropwise from an equilibrating dropping funnel) over a period of ~1 h. The resulting mixture was further stirred for 2 h and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 and chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine (3.1 g, 5.9 mmol, 70%) eluted with 25% hexanes in EtOAc.

Example 46: 2'-Deoxy-2'-Difluoromethylene-Uridine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine (3.1 g, 5.9 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on silica gel column. 2'-Deoxy-2'-difluoromethylene-uridine (1.1 g, 4.0 mmol, 68%) was eluted with 20% MeOH in CH_2Cl_2 .

Example 47: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-Uridine (16) :

2'-Deoxy-2'-difluoromethylene-uridine (1.1 g, 4.0 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (1.42 g, 4.18 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 40% EtOAc:hexanes as eluant to yield 5'-O-DMT-2'-deoxy-2'-difluoromethylene-uridine 16 (1.05 g, 1.8 mmol, 45%).

Example 48: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (18)

1-(2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uracil (0.577 g, 1 mmol) dissolved in dry CH₂Cl₂ (15 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.36 mL, 2 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.44 mL, 1.4 mmol). The reaction mixture was stirred for 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product (0.404 g, 0.52 mmol, 52%) was purified by flash chromatography over silica gel using 20-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.48 (CH₂Cl₂: MeOH / 15:1).

Example 49: 2'-Deoxy-2'-Methylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-4-N-Acetyl-Cytidine 20

Triethylamine (4.8 mL, 34 mmol) was added to a solution of POCl₃ (0.65 mL, 6.8 mmol) and 1,2,4-triazole (2.1 g, 30.6 mmol) in acetonitrile (20 mL) at 0 °C. A solution of 2'-deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl) uridine 19 (1.65 g, 3.4 mmol) in acetonitrile (20 mL) was added dropwise to the above reaction mixture and left to stir at room temperature for 4 h. The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄ concentrated *in vacuo*, dissolved in dioxane (10 mL) and aq.

ammonia (20 mL). The mixture was stirred for 12 h and concentrated *in vacuo*. The residue was azeotroped with anhydrous pyridine (2 x 20 mL). Acetic anhydride (3 mL) was added to the residue dissolved in pyridine, stirred at RT for 4 h and quenched with sat. NaHCO₃ (5 mL). The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄, concentrated *in vacuo* and the residue chromatographed over silica gel. 2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine 20 (1.3 g, 2.5 mmol, 73%) was eluted with 20% EtOAc in hexanes.

10 Example 50: 1-(2'-Deoxy-2'-Methylene-5'-O-Dimethoxytrityl-β-D-ribofuranosyl)-4-N-Acetyl-Cytosine 21

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine 20 (1.3 g, 2.5 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (3 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on silica gel column. 2'-Deoxy-2'-methylene-4-N-acetyl-cytidine (0.56 g, 1.99 mmol, 80%) was eluted with 10% MeOH in CH₂Cl₂. 2'-Deoxy-2'-methylene-4-N-acetyl-cytidine (0.56 g, 1.99 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (0.81 g, 2.4 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃ (50 mL), water (50 mL) and brine (50 mL). The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using EtOAc:hexanes / 60:40 as eluant to yield 21 (0.88 g, 1.5 mmol, 75%).

25 Example 51: 1-(2'-Deoxy-2'-Methylene-5'-O-Dimethoxytrityl-β-D-ribofuranosyl)-4-N-Acetyl-Cytosine 3'-(2-Cyanoethyl-N,N-diisopropylphosphoramidite) (22)

30 1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-4-N-acetyl-cytosine 21 (0.88 g, 1.5 mmol) dissolved in dry CH₂Cl₂ (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.8 mL, 4.5 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.4 mL, 1.8 mmol). The reaction mixture

was stirred 2 h at room temperature and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product 22 (0.82 g, 1.04 mmol, 69%) was purified by flash chromatography over silica gel using 50-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.36 (CH₂Cl₂:MeOH / 20:1).

Example 52: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropyl)disiloxane-1,3-diyl)-4-N-Acetyl-Cytidine (24)

Et₃N (6.9 mL, 50 mmol) was added to a solution of POCl₃ (0.94 mL, 10 mmol) and 1,2,4-triazole (3.1 g, 45 mmol) in acetonitrile (20 mL) at 0 °C. A solution of 2'-deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyl)disiloxane-1,3-diyl)uridine 23 ([described in example 45] 2.6 g, 5 mmol) in acetonitrile (20 mL) was added dropwise to the above reaction mixture and left to stir at RT for 4 h. The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄ concentrated *in vacuo*; dissolved in dioxane (20 mL) and aq. ammonia (30 mL). The mixture was stirred for 12 h and concentrated *in vacuo*. The residue was azeotroped with anhydrous pyridine (2 x 20 mL). Acetic anhydride (5 mL) was added to the residue dissolved in pyridine, stirred at RT for 4 h and quenched with sat. NaHCO₃ (5mL). The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄, concentrated *in vacuo* and the residue chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyl)disiloxane-1,3-diyl)-4-N-acetyl-cytidine 24 (2.2 g, 3.9 mmol, 78%) was eluted with 20% EtOAc in hexanes.

Example 53: 1-(2'-Deoxy-2'-Difluoromethylene-5'-O-Dimethoxytrityl-β-D-ribofuranosyl)-4-N-Acetyl-Cytosine (25)

2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropyl)disiloxane-1,3-diyl)-4-N-acetyl-cytidine 24 (2.2 g, 3.9 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (3 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-difluoromethylene-4-N-acetyl-cytidine (0.89 g, 2.8 mmol, 72%) was eluted with 10% MeOH in CH₂Cl₂. 2'-Deoxy-2'-difluoromethylene-

4-*N*-acetyl-cytidine (0.89 g, 2.8 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (1.03 g, 3.1 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃ (50 mL), water (50 mL) and brine (50 mL). The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using EtOAc:hexanes / 60:40 as eluant to yield 25 (1.2 g, 1.9 mmol, 68%).

10 Example 54: 1-(2'-Deoxy-2'-Difluoromethylene-5'-*O*-Dimethoxytrityl-β-D-ribofuranosyl)-4-*N*-Acetylcytosine 3'-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite) (26)

15 1-(2'-Deoxy-2'-difluoromethylene-5'-*O*-dimethoxytrityl-β-D-ribofuranosyl)-4-*N*-acetylcytosine 25 (0.6 g, 0.97 mmol) dissolved in dry CH₂Cl₂ (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.5 mL, 2.9 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.4 mL, 1.8 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). The product 26, a white foam (0.52 g, 0.63 mmol, 65%) was purified by flash chromatography over silica gel using 30-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.48 (CH₂Cl₂:MeOH / 20:1).

Example 55: 2'-Keto-3',5'-*O*-(Tetraisopropylidisiloxane-1,3-diyl)-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine (28)

25 Acetic anhydride (4.6 mL) was added to a solution of 3',5'-*O*-(tetraisopropylidisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine (Brown, J.; Christodolou, C.; Jones, S.; Modak, A.; Reese, C.; Sibanda, S.; Ubasawa A. *J. Chem. Soc. Perkin Trans. I* 1989, 1735) (6.2 g, 9.2 mmol) in DMSO (37 mL) and the resulting mixture was stirred at room temperature for 24 h. The mixture was concentrated *in vacuo*. The residue was taken up in EtOAc and washed with water. The organic layer was dried over MgSO₄ and concentrated *in vacuo*. The residue was purified on a silica gel column to yield 2'-keto-3',5'-*O*-(tetraisopropylidisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine 28 (4.8 g, 7.2 mmol, 78%).

Example 56: 2'-Deoxy-2'-methylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-Butylbenzoyl)-Adenosine (29)

Under a pressure of argon, sec-butyllithium in hexanes (11.2 mL, 14.6 mmol) was added to a suspension of triphenylmethylphosphonium iodide (7.07 g, 17.5 mmol) in THF (25 mL) cooled at -78 °C. The homogeneous orange solution was allowed to warm to -30 °C and a solution of 2'-keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine 28 (4.87 g, 7.3 mmol) in THF (25 mL) was transferred to this mixture under argon pressure. After warming to RT, stirring was continued for 24 h. THF was evaporated and replaced by CH₂Cl₂ (250 mL), water was added (20 mL), and the solution was neutralized with a cooled solution of 2% HCl. The organic layer was washed with H₂O (20 mL), 5% aqueous NaHCO₃ (20 mL), H₂O to neutrality, and brine (10 mL). After drying (Na₂SO₄), the solvent was evaporated *in vacuo* to give the crude compound, which was chromatographed on a silica gel column. Elution with light petroleum ether:EtOAc / 7:3 afforded pure 2'-deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine 29 (3.86 g, 5.8 mmol, 79%).

Example 57: 2'-Deoxy-2'-Methylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine (3.86 g, 5.8 mmol) dissolved in THF (30 mL) was treated with 1 M TBAF in THF (15 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-methylene-6-N-(4-*t*-butylbenzoyl)-adenosine (1.8 g, 4.3 mmol, 74%) was eluted with 10% MeOH in CH₂Cl₂.

Example 58: 5'-O-DMT-2'-Deoxy-2'-Methylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine (29)

2'-Deoxy-2'-methylene-6-N-(4-*t*-butylbenzoyl)-adenosine (0.75 g, 1.77 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (0.66 g, 1.98 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in

CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 50% EtOAc:hexanes as an eluant to yield 29 (0.81 g, 1.1 mmol, 62%).

5 Example 59: 5'-O-DMT-2'-Deoxy-2'-Methylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine 3'-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (31)

1-(2'-Deoxy-2'-methylene-5'-*O*-dimethoxytrityl-β-D-ribofuranosyl)-6-*N*-(4-*t*-butylbenzoyl)-adenine 29 dissolved in dry CH₂Cl₂ (15 mL) was placed in a round bottom flask under Ar. Diisopropylethylamine was added, followed by
10 the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite. The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). The product was purified by flash chromatography over silica gel using 30-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant (0.7 g, 0.76
15 mmol, 68%). R_f 0.45 (CH₂Cl₂: MeOH / 20:1)

Example 60: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-Butylbenzoyl)-Adenosine

2'-Keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine 28 (6.7 g, 10 mmol) and triphenylphosphine (2.9 g, 11 mmol) were
20 dissolved in diglyme (20 mL), and heated to a bath temperature of 160 °C. A warm (60 °C) solution of sodium chlorodifluoroacetate (2.3 g, 15 mmol) in diglyme (50 mL) was added (dropwise from an equilibrating dropping funnel) over a period of ~1 h. The resulting mixture was further stirred for 2 h and concentrated *in vacuo*. The residue was dissolved in CH₂Cl₂ and
25 chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine (4.1g, 6.4 mmol, 64%) eluted with 15% hexanes in EtOAc.

Example 61: 2'-Deoxy-2'-Difluoromethylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine

30 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine (4.1 g, 6.4 mmol) dissolved in THF (20 mL)

was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-difluoromethylene-6-*N*-(4-*t*-butylbenzoyl)-adenosine (2.3 g, 4.9 mmol, 77%) was eluted with 20% MeOH in CH₂Cl₂.

5 Example 62: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine (30)

2'-Deoxy-2'-difluoromethylene-6-*N*-(4-*t*-butylbenzoyl)-adenosine (2.3 g, 4.9 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 50% EtOAc:hexanes as eluant to yield 30 (2.6 g, 3.41 mmol, 69%).

Example 63: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine 3'-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (32)

1-(2'-Deoxy-2'-difluoromethylene-5'-*O*-dimethoxytrityl-β-D-ribofuranosyl)-6-*N*-(4-*t*-butylbenzoyl)-adenine 30 (2.6 g, 3.4 mmol) dissolved in dry CH₂Cl₂ (25 mL) was placed in a round bottom flask under Ar. Diisopropylethylamine (1.2 mL, 6.8 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (1.06 mL, 4.76 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). 32 (2.3 g, 2.4 mmol, 70%) was purified by flash column chromatography over silica gel using 20-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.52 (CH₂Cl₂: MeOH / 15:1).

Example 64: 2'-Deoxy-2'-Methoxycarbonylmethylidene-3'.5'-*O*-(Tetraiso-propyldisiloxane-1,3-diyl)-Uridine (33)

Methyl(triphenylphosphoranylidene)acetate (5.4 g, 16 mmol) was added to a solution of 2'-keto-3',5'-*O*-(tetraisopropyl disiloxane-1,3-diyl)-uridine 14 in

- CH₂Cl₂ under argon. The mixture was left to stir at RT for 30 h. CH₂Cl₂ (100 mL) and water were added (20 mL), and the solution was neutralized with a cooled solution of 2% HCl. The organic layer was washed with H₂O (20 mL), 5% aq. NaHCO₃ (20 mL), H₂O to neutrality, and brine (10 mL). After drying (Na₂SO₄), the solvent was evaporated *in vacuo* to give crude product, that was chromatographed on a silica gel column. Elution with light petroleum ether:EtOAc / 7:3 afforded pure 2'-deoxy-2'-methoxycarbonylmethylidene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine **33** (5.8 g, 10.8 mmol, 67.5%).

Example 65: 2'-Deoxy-2'-Methoxycarbonylmethylidene-Uridine (34)

- Et₃N•3 HF (3 mL) was added to a solution of 2'-deoxy-2'-methoxycarbonylmethylidene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine **33** (5 g, 9.3 mmol) dissolved in CH₂Cl₂ (20 mL) and Et₃N (15 mL). The resulting mixture was evaporated *in vacuo* after 1 h and chromatographed on a silica gel column eluting 2'-deoxy-2'-methoxycarbonylmethylidene-uridine **34** (2.4 g, 8 mmol, 86%) with THF:CH₂Cl₂ / 4:1.

Example 66: 5'-O-DMT-2'-Deoxy-2'-Methoxycarbonylmethylidene-Uridine (35)

- 2'-Deoxy-2'-methoxycarbonylmethylidene-uridine **34** (1.2 g, 4.02 mmol) was dissolved in pyridine (20 mL). A solution of DMT-Cl (1.5 g, 4.42 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 2-5% MeOH in CH₂Cl₂ as an eluant to yield 5'-O-DMT-2'-deoxy-2'-methoxycarbonylmethylidene-uridine **35** (2.03 g, 3.46 mmol, 86%).

Example 67: 5'-O-DMT-2'-Deoxy-2'-Methoxycarbonylmethylidene-Uridine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (36)

- 1-(2'-Deoxy-2'-2'-methoxycarbonylmethylidene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uridine **35** (2.0 g, 3.4 mmol) dissolved in dry CH₂Cl₂ (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (1.2 mL,

6.8 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.91 mL, 4.08 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). 5'-*O*-DMT-2'-deoxy-2'-methoxycarbonylmethylidene-uridine 3'-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite) **36** (1.8 g, 2.3 mmol, 67%) was purified by flash column chromatography over silica gel using a 30-60% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. *R*_f 0.44 (CH₂Cl₂:MeOH / 9.5:0.5).

Example 68: 2'-Deoxy-2'-Carboxymethylidene-3',5'-*O*-(Tetraisopropylidisiloxane-1,3-diyl)-Uridine **37**

2'-Deoxy-2'-methoxycarbonylmethylidene-3',5'-*O*-(tetraisopropylidisiloxane-1,3-diyl)-uridine **33** (5.0 g, 10.8 mmol) was dissolved in MeOH (50 mL) and 1 N NaOH solution (50 mL) was added to the stirred solution at RT. The mixture was stirred for 2 h and MeOH removed *in vacuo*. The pH of the aqueous layer was adjusted to 4.5 with 1N HCl solution, extracted with EtOAc (2 x 100 mL), washed with brine, dried over MgSO₄ and concentrated *in vacuo* to yield the crude acid. 2'-Deoxy-2'-carboxymethylidene-3',5'-*O*-(tetraisopropylidisiloxane-1,3-diyl)-uridine **37** (4.2 g, 7.8 mmol, 73%) was purified on a silica gel column using a gradient of 10-15% MeOH in CH₂Cl₂.

Example 69: Synthesis of 2'-C-allyl-U phosphoramidite from 5'-*O*-DMT-3'-*O*-TBDMS-Uridine

Referring to Figure 54, in order to simplify the synthetic scheme for phosphoramidites **5** and **8** we also explored the potential of 5'-*O*-DMT-3'-*O*-TBDMS-Uridine **10** (side product in preparation of standard RNA monomers) as a starting material in the synthesis of key intermediate **4**. Phenoxythiocarbonylation of starting synthon **10** according to Robins (Robins, M. J., Wilson J. S. and Hansske, F. (1983), *J. Am. Chem. Soc.*, 105, 4059) surprisingly led to thioester **11** (91 %) without noticeable migration (Scaringe, S.A., Franclyn, C. & Usman, N. (1990) *Nucleic Acids Res.*, 18, 5433-5441) of the TBDMS group. Comparative analysis of ¹H NMR data for compounds **10** and **11** revealed that resonance of H-2' experienced up field shift of 2.0 ppm (from 6.06 to 4.13) in **11** compare to starting compound **10**, at the same time chemical shift of H-3' and H-1' changed only slightly: 4.83 ppm(H-3') and

6.48 ppm (H-1') in 11 compare to 4.36(H-3') ppm and 5.93 ppm (H-1') in 10 and chemical shift of H-4' remains practically unchanged indicating acylation at C-2-OH. Heck allylation of intermediate 11 with 2,2'-Azobis-(2-methyl propionitrile) (other groups can be introduced by standard procedures) resulted in a formation of 2'-C-allyl derivative 12 (70 %) and related 2'-deoxy by-product (15%). Subsequent desilylation of 12 led to 5'-O-DMT derivative 4 identical to the one synthesized from thioester 2. Since the starting material for this route is commercially available this may represent a less laborious way to key synthon 4 as well as for other 2'- modified monomers. This methodology can be used to introduce other 2'-C-allyl groups using compound 11 (or its equivalent for other bases) as an intermediate.

Example 70: Synthesis of 5'-O-Dimethoxytrityl-2'-O-Phenoxythiocarbonyl-3'-O-t-butylidimethylsilyl-uridine 11.

To a stirred solution of 5'-O-Dimethoxytrityl-3'-O-t-butylidimethylsilyl-uridine (Commercially available from Chem Genes Corporation) (5,0 g 7,57 mmol) and dimethylaminopyridine (1,8g, 15 mmol) in 100 ml of dry acetonitrile a solution of phenylchlorothionoformate (1.26ml, 9,1 mmol) in 25 ml of acetonitrile was added dropwise and the reaction mixture stirred at room temperature for 3 hours. TLC (ethylacetate-hexanes 1:1) showed disappearance of starting material and the reaction mixture was concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel CH₂Cl₂ as an eluent to give 5.51g (91.3%) of the product.

¹H NMR (CDCl₃) δ 0.95 (s, 9H, tBu), 0.11 (s, 3H, CH₃), 0.04 (s, 3H, CH₃) 3.57 (2H, H5', H5'', m J_{5',4'}=2.4., J_{5'',4'}=2,8., J_{5',5''}=11.0), 3.86 (6H, OCH₃, s), 4.07 (1H, H4', m), 4.83 (1H, H3', dd, J_{3',4'}=2,8 J_{3',2'}=5,2), 5.44 (1H, H5, d, J_{5,6}=8.0) 5.99 (1H, H2', dd, J_{2',1'}=6.4 , J_{2',3'}= 5,2), 6.46 (1H, H1', d, J_{1',2'}=6.4) , 6.89-7.79 (18H, DMT, Phe, m), 7.88 (1H, H6, d, J_{6,5}=8.0), 7.95 (1H, N-H, bs).

Example 71: Synthesis of 5'-O-Dimethoxytrityl-2'-C-Allyl-3'-O-t-butylidimethylsilyl-uridine(12)

To a refluxing under argon solution of 5'-O-Dimethoxytrityl-2'-O-Phenoxythiocarbonyl-3'-O-t-butylidimethylsilyl-uridine (5,5g, 6,9 mmol) and

allyltributyltin (10,7ml, 34,5 mmol) in dry toluene (150 ml) a solution of 2',2'-Azobis-(2-methyl propionitrile) (0.28g 1,72 mmol) in 50 ml of dry toluene was added dropwise for 1 hour. The resulting mixture was allowed to reflux under argon for additional 2 hours. After that it was concentrated in vacuo and
 5 purified by flash chromatography on silica gel with gradient ethylacetate in hexanes (0-30%) as an eluent. Yield 3.38g (70.0%).

^1H NMR (CDCl_3) δ 0.95 (s, 9H, tBu), 0.11 (s, 3H, CH_3), 0.04 (s, 3H, CH_3), 2.23 (1H, H6', m), 2.38-2.52 (2H, H6'' and H2', m), 3.46 (2H, H5' and H5'', m, $J_{5',4'}=2.5$, $J_{5'',4'}=3.2$, $J_{5',5''}=10.8$), 3.86 (6H, OCH_3 , s), 4.13 (1H, H4', dd, $J_{4',3'}=8.0$, $J_{4',5'}=3.2$, $J_{4',5''}=2.5$), 4.46 (1H, H3', m), 5.15 (1H, H8', d, $J_{8',7'}=10.0$), 5.20 (1H, H9', d, $J_{9',7'}=17.3$), 5.44 (1H, H5, d, $J_{5,6}=8.0$), 5.81 (1H, H7', dddd, $J_{7',6'}=6.0$, $J_{7',6''}=8.0$), 6.14 (1H, H1', d, $J_{1',2'}=8.0$), 6.88-7.52 (13H, DMT, m), 7.76 (1H, H6, d, $J_{6,5}=8.0$), 8.17 (1H, N-H, bs)

Example 72: Synthesis of 5'-O-Dimethoxytrityl-2'-C-Allyl Uridine (4) from 5'-O-Dimethoxytrityl-2'-C-Allyl-3'-O-t-butylidimethyl-silyl-uridine (12).
 15

Standard deprotection of TBDMS derivative 12 utilizing general method A furnished product 4 (yield 80%) identical to the compound prepared from 2'-C-allyl derivative 3.

Uses

20 The alkyl substituted nucleotides of this invention can be used to form stable oligonucleotides as discussed above for use in enzymatic cleavage or antisense situations. Such oligonucleotides can be formed enzymatically using triphosphate forms by standard procedure. Administration of such oligonucleotides is by standard procedure. See Sullivan *et al.* PCT WO
 25 94/02595.

The following are non-limiting examples showing the synthesis of nucleic acids using 2'-O-methylthioalkyl-substituted phosphoramidites and the syntheses of the amidites.

Example 73: Synthesis of Hammerhead Ribozymes Containing 2'-O-alkylthioalkyl nucleotides & Other Modified Nucleotides

The method of synthesis follows the procedure for normal RNA synthesis as described in Usman, N.; Ogilvie, K.K.; Jiang, M.-Y.; Cedergren, R.J. *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and in Scaringe, S.A.; Franklyn, C.; Usman, N. *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. These 2'-O-alkylthioalkyl substituted phosphoramidites may be incorporated not only into hammerhead ribozymes, but also into hairpin, hepatitis delta virus, Group I or Group II intron catalytic nucleic acids, or into antisense oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 74: Synthesis of base-protected 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl) nucleosides (2)

Referring to Figure 55, standard introduction of "Markiewicz" protecting group to the base-protected nucleosides according to "Oligonucleotides and Analogues. A Practical Approach", ed. F. Eckstein, IRL Press, 1991 resulted in protected nucleosides (2) with 85-100% yields. Briefly, in a non-limiting example, Uridine (20g, 81.9 mmol) was dried by two coevaporations with anhydrous pyridine and re dissolved in the anhydrous pyridine. The above solution was cooled (0°C) and solution of 1,3-dichloro-1,1,3,3-tetraisopropylsiloxane (28.82 mL, 90.09 mmol) in 30 mL of anhydrous dichloroethane was added dropwise under stirring. After the addition was completed the reaction mixture was allowed to warm to room temperature and stirred for additional two hours. Then it was quenched with MeOH (25 mL) and evaporated to dryness. The residue was dissolved in methylene chloride and washed with saturated NaHCO₃ and brine. The organic layer was evaporated to dryness and then coevaporated with toluene to remove traces of pyridine to give 39g (98%) of compound 2 (B=Ura) which was used without further purification.

Other 3',5'-O-(tetraisopropylidisiloxane-1,3-di-yl)- nucleosides were obtained in 75-90% yields, using the protocol described above, starting from

base-protected nucleosides with final purification of the products by flash chromatography on silica gel when necessary.

Example 75: General procedure for the synthesis of 2'-O-methylthiomethyl nucleosides (3)

- 5 Referring to Figure 55, to a stirred ice-cooled solution of the mixture of base-protected 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl) nucleoside (2) (7 mmol), methyl disulfide (70 mmol), 2,6-lutidine (7 mmol) in methylene chloride (100 mL) or mixture methylene chloride - acetonitrile (1:1) under positive pressure of argon, solution of benzoyl peroxide (28 mmol) in methylene
10 chloride was added dropwise during 1 hour. After complete addition the reaction mixture was stirred at 0°C under argon for additional 1 hour. The solution was allowed to warm to room temperature, diluted with methylene chloride (100 mL), washed twice with saturated aq NaHCO₃ and brine. The organic layer was dried over sodium sulfate and evaporated to dryness. The
15 residue was purified by flash chromatography on silica using 1-2% methanol in methylene chloride as an eluent to give corresponding methylthiomethyl nucleosides with 55% yield.

Example 76: 5'-O-Dimethoxytrityl-2'-O-Methylthiomethyl-Nucleosides. (6)

- Method A. The solution of the base-protected 3',5'-O-
20 (tetraisopropylidisiloxane-1,3-diyl)-2'-O-methylthiomethyl nucleoside (3) (2.00 mmol) in 10 ml of dry tetrahydrofuran (THF) was treated with 1M solution of tetrabutylammoniumfluoride in THF (3.0 ml) for 10-15 minutes at room temperature. Resulting mixture was evaporated, the residue was loaded to the silica gel column, washed with 1L of chloroform, and the desired
25 deprotected compound was eluted with 5-10% methanol in dichloromethane. Appropriate fractions were combined, solvents removed by evaporation, and the residue was dried by coevaporation with dry pyridine. The oily residue was redissolved in dry pyridine, dimethoxytritylchloride (1.2 eq) was added and the reaction mixture was left under anhydrous conditions overnight. The
30 reaction was quenched with methanol (20 ml), evaporated, dissolved in chloroform, washed with saturated aq sodium bicarbonate and brine. Organic layer was dried over sodium sulfate and evaporated. The residue was purified

by flash chromatography on silica gel to give 5'-O-Dimethoxytrityl derivatives with 70-80% yield.

Method B. Alternatively, 5'-O-Dimethoxytrityl-2'-O-Methylthiomethyl-Nucleosides (6) may also be synthesized using 5'-O-Dimethoxytrityl-3'-O-t-Butyl-dimethylsilyl Nucleosides (4) as the starting material. Compound 4 is commercially available as a by-product during RNA phosphoramidite synthesis. Compound 4 is converted in to 3'-O-t-butyldimethylsilyl-2'-O-methylthiomethyl nucleoside 5, as described under example 3. The solution of the base-protected 3'-O-t-butyldimethylsilyl-2'-O-methylthiomethyl nucleoside 5 (2.00 mmol) in 10 ml of dry tetrahydrofuran (THF) was treated with 1M solution of tetrabutylammoniumfluoride in THF (3.0 ml) for 10-15 minutes at room temperature. The resulting mixture was evaporated, and purified by flash silica gel chromatography to give nucleosides 6 in 90% yield.

Example 77: 5'-O-Dimethoxytrityl-2'-O-Methylthiomethyl-Nucleosides-3'-(2-Cyanoethyl-N,N-diisopropylphosphoramidites) (7)

Standard phosphitylation of nucleoside 6 according to Scaringe, S.A.; Frecklyn, C.; Usman, N. *Nucleic Acids Res.* 1990, 18, 5433-5441 yielded phosphoramidites in 70-85% yield.

Example 78: General procedure for the synthesis of 2'-O-Methylthiophenyl nucleosides.

To a stirred ice-cooled solution of the mixture of base-protected 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl) nucleoside (14.7 mmol), thioanisole (147 mmol), N,N-dimethylaminopyridine (58.8 mmol) in acetonitrile (100 mL) under positive pressure of argon, benzoyl peroxide (36.75 mmol) was added portionwise over 3 hours. After complete addition the reaction mixture was allowed to warm to room temperature and was stirred under argon for an additional 1 hour. The solvents were removed in vacuo, the residue was dissolved in ethylacetate, washed twice with saturated aq NaHCO₃ and brine. The organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by flash chromatography on silica using mixture EtOAc-hexanes (1:1) as eluent to give the corresponding methylthiophenyl nucleosides with 55-65% yield.

Example 79: 5'-O-Dimethoxytrityl-2'-O-Methylthiophenyl-Nucleosides.

These compounds were prepared as described above under examples 76 and 76.

5 Example 80: 5'-O-Dimethoxytrityl-2'-O-Methylthiophenyl-Nucleosides-3'-(2-Cyanoethyl N,N-diisopropylphosphoramidites)

Standard phosphitylation according to Scaringe, S.A.; Franklyn, C.; Usman, N. *Nucleic Acids Res.* 1990, 18, 5433-5441 yielded phosphoramidites in 70-85% yield.

Example 81: Ribozymes containing 2'-O-methylthiomethyl substitutions

10 In a non-limiting example 2'-O-methylthioalkyl substitutions were made at various positions within a hammerhead ribozyme motif (Fig. 56, including U4 and U7 positions). The target site B was targeted by the hammerhead ribozyme in this non-limiting example.

15 Hammerhead ribozymes (see Fig. 56) were synthesized using solid-phase synthesis, as described above. Several positions were modified, individually or in combination, with 2'-O-methylthiomethyl groups.

RNA cleavage assay *in vitro*:

20 Substrate RNA is 5' end-labeled using [γ - 32 P] ATP and T4 polynucleotide kinase (US Biochemicals). Cleavage reactions were carried out under ribozyme "excess" conditions. Trace amount (≤ 1 nM) of 5' end-labeled substrate and 40 nM unlabeled ribozyme are denatured and renatured separately by heating to 90°C for 2 min and snap-cooling on ice for 10-15 min. The ribozyme and substrate are incubated, separately, at 37°C for 10 min in a buffer containing 50 mM Tris-HCl and 10 mM MgCl₂. The reaction is
25 initiated by mixing the ribozyme and substrate solutions and incubating at 37°C. Aliquots of 5 μ l are taken at regular intervals of time and the reaction is quenched by mixing with equal volume of 2X formamide stop mix. The samples are resolved on 20 % denaturing polyacrylamide gels. The results are quantified and percentage of target RNA cleaved is plotted as a function of
30 time.

Referring to Figure 57, hammerhead ribozymes containing 2'-O-methylthiomethyl modifications at various positions cleave the target RNA efficiently. Surprisingly, all the 2'-O-methylthiomethyl-substituted ribozymes cleaved the target RNA more efficiently compared to the control hammerhead
5 ribozyme.

Sequences listed in Figure 56 and the modifications described in Figure 56 and 57 are meant to be non-limiting examples. Those skilled in the art will recognize that variants (base-substitutions, deletions, insertions, mutations, chemical modifications) of the ribozyme and RNA containing other
10 combinations of 2'-hydroxyl group modifications can be readily generated using techniques known in the art, and are within the scope of the present invention.

The following are non-limiting examples showing the synthesis of non-nucleotide mimetic-containing catalytic nucleic acids using non-nucleotide
15 phosphoramidites.

Such non-nucleotides can be located in the binding arms, core or the loop adjacent stem II of a hammerhead type ribozyme. Those in the art following the teachings herein can determine optimal locations in these regions. Surprisingly, abasic moieties can be located in the core of such a
20 ribozyme.

Example 82: Synthesis of Abasic nucleotides

The synthesis of 1-deoxy-D-ribofuranose phosphoramidite **9** is shown in Figure 58. Our initial efforts concentrated on the deoxygenation of synthon **1**, prepared by a "one pot" procedure from D-ribose. Phenoxythiocarbonylation of acetonide **1** under Robins conditions led to the β -anomer **2** ($J_{1,2} = 1.2$ Hz) in modest yield (45-55%). Radical deoxygenation using $\text{Bu}_3\text{SnH/AIBN}$ resulted in the formation of the ribitol derivative **3** in 50% yield. Subsequent deprotection with 90% CF_3COOH (10 m) and introduction of a dimethoxytrityl group led to the key intermediate **4** in 40% yield (Yang et al., *Biochemistry*
25 1992, 31, 5005-5009; Perreault et al., *Biochemistry* 1991, 30, 4020-4025; Paoletta et al., *EMBO J.* 1992, 11, 1913-1919; Peiken et al., *Science* 1991, 253, 314-317).

The low overall yield of this route prompted us to investigate a different approach to 4 (Fig. 58). Phenylthioglycosides, successfully employed in the Keck reaction, appeared to be an alternative. However, it is known that free-radical reduction of the corresponding glycosyl bromides with participating acyl groups at the C2-position can result in the migration of the 2-acyl group to the C1-position (depending on Bu_3SnH concentration). Therefore we subjected phenylthioglycoside 5 to radical reduction with Bu_3SnH (6.1 eq.) in the presence of Bz_2O_2 (2 eq.) resulting in the isolation of tribenzoate 6 in 63% yield (Fig. 9B). Subsequent debenzoylation and dimethoxytritylation led to synthon 4 in 70% yield. Introduction of the TBDMS group, using standard conditions, resulted in the formation of a 4:1 ratio of 2- and 3-isomers 8 and 7. The two regioisomers were separated by silica gel chromatography. The 2-O-*t*-butyldimethylsilyl derivative 8 was phosphitylated to provide phosphoramidite 9 in 82% yield.

15 Example 83: RNA cleavage assay *in vitro*

Ribozymes and substrate RNAs were synthesized as described above. Substrate RNA was 5' end-labeled using [γ - ^{32}P] ATP and T4 polynucleotide kinase (US Biochemicals). Cleavage reactions were carried out under ribozyme "excess" conditions. Trace amount (≤ 1 nM) of 5' end-labeled substrate and 40 nM unlabeled ribozyme were denatured and renatured separately by heating to 90°C for 2 min and snap-cooling on ice for 10 -15 min. The ribozyme and substrate were incubated, separately, at 37°C for 10 min in a buffer containing 50 mM Tris-HCl and 10 mM MgCl_2 . The reaction was initiated by mixing the ribozyme and substrate solutions and incubating at 37°C. Aliquots of 5 μl are taken at regular intervals of time and the reaction quenched by mixing with an equal volume of 2X formamide stop mix. The samples were resolved on 20 % denaturing polyacrylamide gels. The results were quantified and percentage of target RNA cleaved is plotted as a function of time.

30 Referring to Figure 59 there is shown the general structure of a hammerhead ribozyme targeted against site B (HH-B) with various bases numbered. Various substitutions were made at several of the nucleotide positions in HH-B. Specifically referring to Figure 60, substitutions were made

at the U4 and U7 positions marked as X4 and X7 and also in loop II in the positions marked by an X. The RNA cleavage activity of these substituted ribozymes is shown in the following figures. Specifically, Figure 61 shows cleavage by an abasic substituted U4 and an abasic substituted U7. As will be noted, abasic substitution at U4 or U7 does not significantly affect cleavage activity. In addition, inclusion of all abasic moieties in stem II loop does not significantly reduce enzymatic activity as shown in Figure 62. Further, inclusion of a 3' inverted deoxyribose does not inactivate the RNA cleavage activity as shown in Figure 63.

10 Example 84: Smooth Muscle Cell Proliferation Assay

Hammerhead ribozyme (HH-A) is targeted to a unique site (site A) within *c-myb* mRNA. Expression of *c-myb* protein has been shown to be essential for the proliferation of rat smooth muscle cell (Brown et al., 1992 *J. Biol. Chem.* 267, 4625).

15 The ribozymes that cleaved site A within *c-myb* RNA described above were assayed for their effect on smooth muscle cell proliferation. Rat vascular smooth muscle cells were isolated and cultured as described (Stnchcomb et al., *supra*). These primary rat aortic smooth muscle cells (RASMC) were plated in a 24-well plate (5×10^3 cells/well) and incubated at 37°C in the presence of Dulbecco's Minimal Essential Media (DMEM) and 10% serum for -16 hours.

25 These cells were serum-starved for 48-72 hours in DMEM (containing 0.5% serum) at 37°C. Following serum-starvation, the cells were treated with lipofectamine (LFA)-complexed ribozymes (100 nM ribozyme was complexed with LFA such that LFA:ribozyme charge ration is 4:1).

30 Ribozyme:LFA complex was incubated with serum-starved RASMC cells for four hours at 37°C. Following the removal of ribozyme:LFA complex from cells (after 4 hours), 10% serum was added to stimulate smooth cell proliferation. Bromo-deoxyuridine (BrdU) was added to stain the cells. The cells were stimulated with serum for 24 hours at 37°C.

Following serum-stimulation, RASMC cells were quenched with hydrogen peroxide (0.3% H₂O₂ in methanol) for 30 min at 4°C. The cells were then denatured with 0.5 ml 2N HCl for 20 min at room temperature. Horse serum (0.5 ml) was used to block the cells at 4°C for 30 min up to ~16 hours.

The RASMC cells were stained first by treating the cells with anti-BrdU (primary) antibody at room temperature for 60 min. The cells were washed with phosphate-buffered saline (PBS) and stained with biotinylated affinity-purified anti-mouse IgM (Pierce, USA) secondary antibody. The cells were counterstained using avidin-biotinylated enzyme complex (ABC) kit (Pierce, USA).

The ratio of proliferating:non-proliferating cells was determined by counting stained cells under a microscope. Proliferating RASMCs will incorporate BrdU and will stain brown. Non-proliferating cells do not incorporate BrdU and will stain purple.

Referring to Figure 64 there is shown a ribozyme which cleaves the site A referred to as HH-A. Substitutions of abasic moieties in place of U4 as shown in Figure 65 provided active ribozyme as shown in Figure 66 using the above-noted rat aortic smooth muscle cell proliferation assay.

The method of this invention generally features HPLC purification of ribozymes. An example of such purification is provided below in which a synthetic ribozyme produced on a solid phase is blocked. This material is then released from the solid phase by a treatment with methanolic ammonia, subsequently treated with tetrabutylammonium fluoride, and purified on reverse phase HPLC to remove partially blocked ribozyme from "failure" sequences. Such "failure" sequences are RNA molecules which have a nucleotide base sequence shorter to that of the desired enzymatic RNA molecule by one or more of the desired bases in a random manner, and possess free terminal 5'-hydroxyl group. This terminal 5'-hydroxyl in a ribozyme with the correct sequence is still blocked by lipophilic dimethoxytrityl group. After such partially blocked enzymatic RNA is purified, it is deblocked by a standard procedure, and passed over the same or a similar HPLC

reverse phase column to remove other contaminating components, such as other RNA molecules or nucleotides or other molecules produced in the deblocking and synthetic procedures. The resulting molecule is the native enzymatically active ribozyme in a highly purified form.

- 5 Below are provided examples of such a method. These examples can be readily scaled up to allow production and purification of gram or even kilogram quantities of ribozymes.

Example 85: HPLC Purification, Reverse-Phase

- 10 In this example solid phase phosphoramidite chemistry was employed for synthesis of a ribozyme. Monomers used were 2'-*t*-butyl-dimethylsilyl cyanoethylphosphoramidites of uridine, *N*-benzoyl-cytosine, *N*-phenoxyacetyl adenosine, and guanosine (Glen Research, Sterling, VA).

- 15 Solid phase synthesis was carried out on either an ABI 394 or 380B DNA/RNA synthesizer using the standard protocol provided with each machine. The only exception was that the coupling step was increased from 10 to 12 minutes. The phosphoramidite concentration was 0.1 M. Synthesis was done on a 1 μ mol scale using a 1 μ mol RNA reaction column (Glen Research). The average coupling efficiencies were between 97% and 98% for the 394 model and between 97% and 99% for the 380B model, as
20 determined by a calorimetric measurement of the released trityl cation. The final 5'-DMT group was not removed.

- 25 After synthesis, the ribozymes were cleaved from the CPG support, and the base and phosphotriester moieties were deprotected in a sterile vial by incubation in dry ethanolic ammonia (2 mL) at 55 °C for 16 hours. The reaction mixture was cooled on dry ice. Later, the cold liquid was transferred into a sterile screw cap vial and lyophilized.

- 30 To remove the 2'-*t*-butyldimethylsilyl groups from the ribozyme the obtained residue was suspended in 1 M tetra-*n*-butylammonium fluoride in dry THF (TBAF), using a 20-fold excess of the reagent for every silyl group, for 16 hours at ambient temperature. The reaction was quenched by adding an

equal volume of a sterile 1 M triethylamine acetate, pH 6.5. The sample was cooled and concentrated on a SpeedVac to half of the initial volume.

The ribozymes were purified in two steps by HPLC on a C4 300 Å 5 µm DeltaPak column in an acetonitrile gradient.

5 The first step, or "trityl on" step, was a separation of 5'-DMT-protected ribozyme(s) from failure sequences lacking a 5'-DMT group. Solvents used for this step were: A (0.1 M triethylammonium acetate, pH 6.8) and B (acetonitrile). The elution profile was: 20% B for 10 minutes, followed by a linear gradient of 20% B to 50% B over 50 minutes, 50% B for 10 minutes, a
10 linear gradient of 50% B to 100% B over 10 minutes, and a linear gradient of 100% B to 0% B over 10 minutes.

15 The second step was a purification of a completely deprotected, *i.e.* following the removal of the 5'-DMT group, ribozyme by a treatment with 2% trifluoroacetic acid or 80% acetic acid on a C4 300 Å 5 µm DeltaPak column in an acetonitrile gradient. Solvents used for this second step were: A (0.1 M Triethylammonium acetate, pH 6.8) and B (80% acetonitrile, 0.1 M triethylammonium acetate, pH 6.8). The elution profile was: 5% B for 5 minutes, a linear gradient of 5% B to 15% B over 60 minutes, 15% B for 10 minutes, and a linear gradient of 15% B to 0% B over 10 minutes.

20 The fraction containing ribozyme, which is in the triethylammonium salt form, was cooled and lyophilized on a SpeedVac. Solid residue was dissolved in a minimal amount of ethanol and ribozyme in sodium salt form was precipitated by addition of sodium perchlorate in acetone. (K^+ or Mg^{2+} salts can be produced in an equivalent manner.) The ribozyme was collected
25 by centrifugation, washed three times with acetone, and lyophilized.

Example 86: RNA and Ribozyme Deprotection of Exocyclic Amino Protecting Groups Using ethylamine (EA)

30 The polymer-bound oligonucleotide, either trityl-on or off, was suspended in a solution of ethylamine (EA) @ 25-55 °C for 10-30 min to remove the exocyclic amino protecting groups (see Figure 67). The supernatant was removed from the polymer support. The support was washed with 1.0 mL of

EtOH:MeCN:H₂O/3:1:1, vortexed and the supernatant was then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, were dried to a white powder.

5 Table EVII is a summary of the results obtained using the improvements outlined in this application for base deprotection. From this data it is evident EA at 55° for 10 m or 40° for 10 m is efficient. The HPLC peak structure is almost identical between these schemes, and the yield for the ethylamine deprotected oligos is actually slightly better than the methylamine.

10 The second step of the deprotection of RNA molecules may be accomplished by removal of the 2'-hydroxyl alkylsilyl protecting group using TBAF for 8-24 h (Usman *et al.* *J. Am. Chem. Soc.* 1987, 109, 7845-7854). Applicant has determined that the use of anhydrous TEA•HF in *N*-methylpyrrolidine (NMP) for 0.5-1.5 h @ 55-65 °C gives equivalent or better results.

15 The following are examples of preferred embodiments of the present invention. Those in the art will recognize that these are not limiting examples but rather are provided to guide those in the art to the full breadth of meaning of the present invention. Routine procedures can be used to utilize other coupling regions not exemplified below.

20 Ribozymes were synthesized in two parts and tested without ligation for catalytic activity. Referring to Fig. 72, the cleavage activity of the half ribozymes containing between 5 and 8 base pairs stem IIs at 40 nM under single turnover conditions was comparable to that of the full length oligomer as shown in Figs. 73 and 74. The same half ribozymes were synthesized with
25 suitable modifications at the nascent stem II loop to allow for crosslinking. The halves were purified and chemically ligated, using a variety of crosslinking methods. The resulting full length ribozymes (see Fig. 71) exhibited similar cleavage activity as the linearly synthesized full length oligomer as shown in Fig. 74.

Example 87

Referring to Fig. 70 the 5' half of a hammerhead ribozyme was provided with a ribose group. This was oxidatively cleaved with NaIO_4 and reacted with the 3' half of the ribozyme having an amino group under reducing conditions.

- 5 The resulting ribozyme consisted of the two half ribozyme linked by a morpholino group.

One equivalent of (200 micrograms) of 5' half hammerhead with a 3'OH and 5 equivalents (1000 micrograms) of 3' half with 5' C5-NH₂ all with HH-A were used in this reaction. The limiting oligonucleotide was oxidized first with
10 3.6 equivalents of sodium periodate for sixty minutes on ice in DEPC water quenched with 7.2 equivalents of ethylene glycol for 30 minutes on ice and the 5 equivalents of the amino oligo added. 0.5 Molar tricine buffer, pH 9, was added to provide 25 millimolar final tricine concentration and left for 30 minutes on ice. 50 equivalents of sodium cyanoborohydride was then added
15 and the pH reduced to 6.5 with acetic acid and reaction left for 60 minutes on ice. The resulting full length ribozyme was then purified for further analysis.

Example 88: Amide Bond

Referring again to Fig. 70 and 71, a 5' half of ribozyme was provided with a carboxyl group at its 2' position and was coupled with an amine containing
20 3' half ribozyme. The provision of a coupling reagent resulted in a full-length ribozyme having an amide bond.

Example 89: Disulfide Bond

Referring to Fig. 70 and 71, 250 micrograms of RPI3881 and 250 micrograms of RPI3636 half ribozyme were separately deprotected with
25 dithiothreitol overnight at 37°C. They were mixed together at 1:1 mole ratio in a 100 mM sodium phosphate buffer at pH 8 and 4M copper sulfate and 0.8 mM 1,10-phenanthroline (final concentrations) was added for two hours at room temperature (20-25°C) and the resulting mixture gel purified. The overall purification yield of full length ribozyme was 30%.

30 To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 1.8 KB region (containing site A) was synthesized by PCR using

primers that place the T7 RNA promoter upstream of the amplified sequence. Target RNA was transcribed, using T7 RNA polymerase, in a standard transcription buffer in the presence of [α - 32 P]CTP. The reaction mixture was treated with 15 units of ribonuclease-free DNaseI, extracted with phenol
5 followed chloroform:isoamyl alcohol (25:1), precipitated with isopropanol and washed with 70% ethanol. The dried pellet was resuspended in 20 μ l DEPC-treated water and stored at -20°C.

Unlabeled ribozyme (200 nM) and internally labeled 1.8 KB substrate RNA (<10 nM) were denatured and renatured separately in a standard
10 cleavage buffer (containing 50 mM Tris-HCl pH 7.5 and 10 mM MgCl₂) by heating to 90°C for 2 min. and slow cooling to 37°C for 10 min. The reaction was initiated by mixing the ribozyme and substrate mixtures and incubating at 37°C. Aliquots of 5 μ l were taken at regular time intervals, quenched by adding an equal volume of 2X formamide gel loading buffer and frozen on dry
15 ice. The samples were resolved on 5% polyacrylamide sequencing gel and results were quantitatively analyzed by radioanalytic imaging of gels with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Few antiviral drug therapies are available that effectively inhibit established viral infections. Consequently, prophylactic immunization has
20 become the method of choice for protection against viral pathogens. However, effective vaccines for divergent viruses such as those causing the common cold, and HIV, the etiologic agent of AIDS, may not be feasible. Consequently, new antiviral strategies are being developed for combating viral infections.

25 Gene therapy represents a potential alternative strategy, where antiviral genes are stably transferred into susceptible cells. Such gene therapy approaches have been termed "intracellular immunization" since cells expressing antiviral genes become immune to viral infection (Baltimore, 1988 *Nature* 335, 395-396). Numerous forms of antiviral genes have been
30 developed, including protein-based antivirals such as transdominant inhibitory proteins (Malim et al., 1993 *J. Exp. Med.*, Bevec et al., 1992 *P.N.A.S. (USA)* 89, 9870-9874; Bahner et al., 1993 *J. Virol.* 67, 3199-3207) and viral-activated suicide genes (Ashorn et al., 1990 *P.N.A.S.(USA)* 87, 8889-8893). Although

effective in tissue culture, protein-based antivirals have the potential to be immunogenic *in vivo*. It is therefore conceivable that treated cells expressing such foreign antiviral proteins will be eradicated by normal immune functions. Alternatives to protein based antivirals are RNA based molecules such as antisense RNAs, decoy RNAs, agonist RNAs, antagonist RNAs, therapeutic editing RNAs and ribozymes. RNA is not immunogenic; therefore, cells expressing such therapeutic RNAs are not susceptible to immune eradication.

Example 90: Design and construction of U6-S35 Chimera

A transcription unit, termed U6-S35, is designed that contains the characteristic intramolecular stem of a S35 motif (see Figure 76). As shown in Figure 77, 78 and 79 a desired RNA (e.g. ribozyme) can be inserted into the indicated region of U6-S35 chimera. This construct is under the control of a type 3 pol III promoter, such as a mammalian U6 small nuclear RNA (snRNA) promoter (see Fig. 75). U6-S35-HHI and U6-S35-HHII are non-limiting examples of the U6-S35 chimera.

As a non-limiting example, applicant has constructed a stable, active ribozyme RNA driven from a eukaryotic U6 promoter (Fig. 78). For stability, applicant incorporated a S35 motif as described in Fig. 76 and Fig. 77. A ribozyme sequence is inserted at the top of the stem, such that the ribozyme is separated from the S35 motif by an unstructured spacer sequence (Fig. 77, 78, 79). The spacer sequence can be customized for each desired RNA sequence. U6-S35 chimera is meant to be a non-limiting example and those skilled in the art will recognize that the structure disclosed in the figures 77, 78 and 79 can be driven by any of the known RNA polymerase promoters and are within the scope of this invention. All that is necessary is for the 5' region of a transcript to interact with its 3' region to form a stable intramolecular structure (S35 motif) and that the S35 motif is separated from the desired RNA by a stretch of unstructured spacer sequence. The spacer sequence appears to improve the effectiveness of the desired RNA.

By "unstructured" is meant lack of a secondary and tertiary structure such as lack of any stable base-paired structure within the sequence itself, and preferably with other sequences in the attached RNA.

By "spacer sequence" is meant any unstructured RNA sequence that separates the S35 domain from the desired RNA. The spacer sequence can be greater than or equal to one nucleotide.

In vitro Catalytic Activity of U6-S35-Ribozyme Chimeras:

5 U6-S35-HHI ribozyme RNA was synthesized using T7 RNA polymerase. HHI RNA was chemically synthesized using RNA phosphoramidite chemistry as described in Wincott et al., 1995 *Nucleic Acids Res.* The ribozyme RNAs were gel-purified and the purified ribozyme RNAs were heated to 55°C for 5 min. Target RNA used was ~650 nucleotide long. Internally-³²P-labeled
10 target RNA was prepared as described above. The target RNA was pre-heated to 37°C in 50 mM Tris.HCl, 10 mM MgCl₂ and then mixed at time zero with the ribozyme RNAs (to give 200 nM final concentration of ribozyme). At appropriate times an aliquot was removed and the reaction was stopped by dilution in 95% formamide. Samples were resolved on a denaturing urea-
15 polyacrylamide gel and products were quantitated on a phosphorimager®.

As shown in Figure 80, the U6-S35-HHI ribozyme chimera cleaved its target RNA as efficiently as a chemically synthesized HHI ribozyme. In fact, it appears that the U6-S35-HHI ribozyme chimera may be more efficient than the synthetic ribozyme.

20 *Accumulation of U6-S35-ribozyme transcripts*

An Actinomycin D assay was used to measure accumulation of the transcript in mammalian cells. Cells were transfected overnight with plasmids encoding the appropriate transcription units (2µg DNA/well of 6 well plate) using calcium phosphate precipitation method (Maniatis et al., 1982 *Molecular*
25 *Cloning* Cold Spring Harbor Laboratory Press, NY). After the overnight transfection, media was replaced and the cells were incubated an additional 24 hours. Cells were then incubated in media containing 5µg/ml Actinomycin D. At the times indicated, cells were lysed in guanidinium isothiocyanate, and total RNA was purified by phenol/chloroform extraction and isopropanol
30 precipitation as described by Chomczynski and Sacchi, 1987 *Anal. Biochem.*, 162, 156. RNA was analyzed by northern blot analysis and the levels of

specific RNAs were radioanalytically quantitated on a phosphorimager®. The level of RNA at time zero was set to be 100%.

As shown in Figure 81, the U6-S35-HHII ribozyme shown in Figure 79 is fairly stable in 293 mammalian cells with an approximate half-life of about 2 hours.

Example 91: Design and construction of VA1-S35 Chimera

Referring to Figure 83A, In order to express ribozymes from a VAI promoter, applicant has constructed a transcription unit consisting of a wild type VA1 sequence with two modifications: a "S35-like" motif extends from a loop in the central domain (Figure 82); the 3' terminus is changed such that there is a more complete interaction between the 5' and the 3' region of the transcript (specifically, an "A-C" bulge is changed to an "A-U base pair and the termination sequence is part of the stem of S35 motif).

Accumulation of VA1-S35-ribozyme transcripts

An Actinomycin D assay was used to measure accumulation of the transcript in mammalian cells as described above. As shown in Figure 84, the VA1-S35-chimera, shown in Figure 83A, has approximately 10-fold higher stability in 293 mammalian cells compared to VA1-chimera, shown in Figure 25B that lacks the intramolecular S35 motif.

Besides ribozymes, desired RNAs like antisense, therapeutic editing RNAs, decoys, can be readily inserted into the indicated U6-S35 or VA1-S35 chimera to achieve therapeutic levels of RNA expression in mammalian cells.

Sequences listed in the Figures are meant to be non-limiting examples. Those skilled in the art will recognize that variants (mutations, insertions and deletions) of the above examples can be readily generated using techniques known in the art, are within the scope of the present invention.

Diagnostic uses

Ribozymes of this invention may be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of

stromolysin, B7-1, B7-2, B7-3 and/or CD40 or other RNAs in a cell. The close relationship between ribozyme activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple ribozymes described in this invention, one may map nucleotide changes which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combinational therapies (e.g., multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other *in vitro* uses of ribozymes of this invention are well known in the art, and include detection of the presence of mRNAs associated with B7-1, B7-2, B7-3 and/or CD40 or other RNA related conditions. Such RNA is detected by determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

In a specific example, ribozymes which can cleave only wild-type or mutant forms of the target RNA are used for the assay. The first ribozyme is used to identify wild-type RNA present in the sample and the second ribozyme will be used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA will be cleaved by both ribozymes to demonstrate the relative ribozyme efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates will also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus each analysis will require two ribozymes, two substrates and one unknown sample which will be combined into six reactions. The presence of cleavage products will be determined using an RNase protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and

- putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (*i.e.*, B7-1, B7-2, B7-3 and/or CD40) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a
- 5 qualitative comparison of RNA levels will be adequate and will decrease the cost of the initial diagnosis. Higher mutant form to wild-type ratios will be correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

Other embodiments are within the following claims.

TABLE I**Characteristics of Ribozymes****Group I Introns**

Size: ~200 to >1000 nucleotides.

Requires a U in the target sequence immediately 5' of the cleavage site.

Binds 4-6 nucleotides at 5' side of cleavage site.

Over 75 known members of this class. Found in *Tetrahymena thermophila* rRNA, fungal mitochondria, chloroplasts, phage T4, blue-green algae, and others.

RNAseP RNA (M1 RNA)

Size: ~290 to 400 nucleotides.

RNA portion of a ribonucleoprotein enzyme. Cleaves tRNA precursors to form mature tRNA.

Roughly 10 known members of this group all are bacterial in origin.

Hammerhead Ribozyme

Size: ~13 to 40 nucleotides.

Requires the target sequence UH immediately 5' of the cleavage site.

Binds a variable number nucleotides on both sides of the cleavage site.

14 known members of this class. Found in a number of plant pathogens (virusoids) that use RNA as the infectious agent (Figure 1)

Hairpin Ribozyme

Size: ~50 nucleotides.

Requires the target sequence GUC immediately 3' of the cleavage site.

Binds 4-6 nucleotides at 5' side of the cleavage site and a variable number to the 3' side of the cleavage site.

Only 3 known member of this class. Found in three plant pathogen (satellite RNAs of the tobacco ringspot virus, arabis mosaic virus and chicory yellow mottle virus) which uses RNA as the infectious agent (Figure 3).

Hepatitis Delta Virus (HDV) Ribozyme

Size: 50 - 60 nucleotides (at present).

Cleavage of target RNAs recently demonstrated.

Sequence requirements not fully determined.

Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required.

Only 1 known member of this class. Found in human HDV (Figure 4).

***Neurospora* VS RNA Ribozyme**

Size: ~144 nucleotides (at present)

Cleavage of target RNAs recently demonstrated.

Sequence requirements not fully determined.

Binding sites and structural requirements not fully determined. Only 1 known member of this class. Found in *Neurospora* VS RNA (Figure 5).

Table AII: Human Stromelysin Hammerhead Target Sequence

<u>nt</u> <u>Position</u>	<u>Sequence</u>	<u>SEQ. ID. NO.</u>
20	UAGAGCUAAGUAAAGCCAG	ID. NO. 01
126	ACACCAGCAUGAA	ID. NO. 02
147	AGAAAUUUCUAGA	ID. NO. 03
171	ACCUCAAAAAAGAUUGUGAAACAGU	ID. NO. 04
240	AAUUGCAGAAGUUC	ID. NO. 05
287	GACACUCUGGAGGUGAUGGCGCAAGCCCAGGUGU	ID. NO. 06
327	CUGAUGUUGGUCACUUCAGAAC	ID. NO. 07
357	GCAUCCCGAAGUGGAGGAAAAACCCACCUUACAU	ID. NO. 08
402	AUUUAUACACCAGAUUUGCCAAAAGAU	ID. NO. 09
429	CUGUUGAUUCUGCUGUUGAGA	ID. NO. 10
455	CUGAAAGUCUGGGAAGAGGUGA	ID. NO. 11
513	CUGAUUAUAUGA	ID. NO. 12
592	UGCCU AUGCCCC	ID. NO. 13
624	AUGCCCAUUUGAUGAUGAUGAACAAUGGACA	ID. NO. 14
671	AUUUCUUGUUGCUGCUCAUG	ID. NO. 15
725	CACUCAGCCAACACUGA	ID. NO. 16
801	AAGAUGAUUAUAAUUGGCAUUCAGUCC	ID. NO. 17
827	CUCUAUGGACCUCCCCUGACUCCCCU	ID. NO. 18
859	CCCCUGGUACCCA	ID. NO. 19
916	UCCUGCUUUGUCCUUGAUGCUGUCAGCAC	ID. NO. 20
958	AAUCCUGAUCUUUAAAGA	ID. NO. 21
975	CAGGCACUUUUGGCGCAAUCCC	ID. NO. 22
1018	AUUGCAUUUGAUCUCUUCAUUUUGGCCAUC	ID. NO. 23
1070	GCAUAUGAAGUUA	ID. NO. 24
1203	AAAUUGAUGCAGCCAUUUCUGA	ID. NO. 25
1274	UUUGAUGAGAAGAGAAAUUCCAUUGGAGC	ID. NO. 26
1302	CAGGCUUCCCCAAGCAAUAGCUGAAGAC	ID. NO. 27
1420	CCCAAUUGCAAAG	ID. NO. 28
1485	AUGUAGAAGGCACAUAUUGGCACUUUAAA	ID. NO. 29
1623	UCUUGCCGGUCAUUUUAUGUUAU	ID. NO. 30
1665	GCUGCUGCUUAGC	ID. NO. 31

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1733

CAACAGACAAGUGACUGUAUCU

ID. NO. 32

1769

CUAUAUAUA

ID. NO. 33

Table AIII: Human Stromelysin HH Target Sequence

nt. Position	Target Sequence	Seq. ID. NO.
10	GCAAGGCAUA GAGACAACAUAGAGC	ID. NO. 34
21	GCAUAGAGACAACAUUA GAGCUAAGUAAAGCC	ID. NO. 35
27	AGACAACAUAGAGCUA AGUAAAGCCAGUGGA	ID. NO. 36
31	AACAUAGAGCUAAGUA AAGCCAGUGGAAAU	ID. NO. 37
53	GUGGAAUGAAGAGUC UUCCA AUCCUACUGU	ID. NO. 38
55	GGAAALGAAGAGUCUU CCAUCCUACUGUUG	ID. NO. 39
56	GAAALGAAGAGUCUUC CAUCCUACUGUUGC	ID. NO. 40
61	GAAGAGUCUCCAUC CUACUGUUGCUGUUC	ID. NO. 41
64	GAGUCUCCA AUCCUA CUGUUGCUGUGUG	ID. NO. 42
69	UUCCA AUCCUACUGU GCUGUGGUGGCAGU	ID. NO. 43
85	GCUGUGGUGGCAGUU UGCUCAGCCUAUCCA	ID. NO. 44
86	CUG UUGGCAGUUU GCUCAGCCUAUCCAU	ID. NO. 45
90	GUGGUGGAGUUUGUC AGCCUAUCCAUUGA	ID. NO. 46
96	CAGUUUGCUCAGCCUA UCCAUUGGAUGGAGC	ID. NO. 47
98	GUUUGCUCAGCCUAC CAUUGGAUGGAGCUG	ID. NO. 48
102	GCUCAGCCUAUCCAUU GGAUGGAGCUGCAAG	ID. NO. 49
142	CACCAGCAUGAACCUU GUUCAGAAAUUUCUA	ID. NO. 50
145	CAGCAUGAACCUUGUU CAGAAAUUUCUAGAA	ID. NO. 51
146	AGCAUGAACCUUGUUC AGAAAUUUCUAGAAA	ID. NO. 52
153	ACCUUGUUCAGAAAUU UCUAGAAAACUACUA	ID. NO. 53
155	CUUGUUCAGAAAUUC UAGAAAACUACUACG	ID. NO. 54
157	UGUUCAGAAAUUUCUA GAAAACUACUACGAC	ID. NO. 55
165	AAUUAUCUAGAAAACUA CUACGACCUCAAAAA	ID. NO. 56
168	AUCUAGAAAACUACUA CGACCUCAAAAAAGA	ID. NO. 57
175	AAACUACUACGACCUC AAAAAAGAUUGGAAA	ID. NO. 58
195	AAGAUGUGAAACAGUU UGUUAGGAGAAAGGA	ID. NO. 59
196	AGAUGUGAAACAGUUU GUUAGGAGAAAGGAC	ID. NO. 60

199	UGUGAAACAGUUGUU AGGAGAAAGGACAGU	ID. NO. 61
200	GUGAAACAGUUGUUA GGAGAAAGGACAGUG	ID. NO. 62
218	AGAAAGGACAGUGGUC CUGUUGUUAUUUUUU	ID. NO. 63
223	GGACAGUGGUCCUGUU GUUAAAAAUAUUGA	ID. NO. 64
226	CAGUGGUCCUGUUGUU AAAAAAUAUUGAGAA	ID. NO. 65
227	AGUGGUCCUGUUGUUA AAAAAAUAUUGAGAAA	ID. NO. 66
235	UGUUGUUAUUUUUAUC CGAGAAUUGCAGAAG	ID. NO. 67
252	GAGAAUUGCAGAAGUU CCUUGGAUUGGAGGU	ID. NO. 68
253	AGAAUUGCAGAAGUUC CUUGGAUUGGAGGUG	ID. NO. 69
256	AAUUGCAGAAGUCCUU GGAUUGGAGGUGACG	ID. NO. 70
261	AGAAGUUCUUGGAUU GGAGGUGACGGGGAA	ID. NO. 71
285	CGGGGAGCUGGACUC CGACACUCUGGAGGU	ID. NO. 72
293	CUGGACUCUGACACUC UGGAGGUGAUGGCGA	ID. NO. 73
325	GCCCAGGUGUGGAGUU CCUGAUGUUGGUCAC	ID. NO. 74
326	CCCAGGUGUGGAGUUC CUGAUGUUGGUCACU	ID. NO. 75
334	UGGAGUUCUGAUGUU GGUCACUUCAGAACC	ID. NO. 76
338	GUUCCUGAUGUUGGUC ACUUCAGAACCUUUC	ID. NO. 77
342	CUGAUGUUGGUCACUU CAGAACCUUUCUUGG	ID. NO. 78
343	UGAUGUUGGUCACUUC AGAACCUUUCUUGGC	ID. NO. 79
351	GUCACUUCAGAACCUU UCCUGGCAUCCCGAA	ID. NO. 80
352	UCACUUCAGAACCUU CCUGGCAUCCCGAAG	ID. NO. 81
353	CACUUCAGAACCUUUC CUGGCAUCCCGAAGU	ID. NO. 82
361	AACCUUUCUUGGCAUC CCGAAGUGGAGGAAA	ID. NO. 83
385	GAGGAAAACCCACCUU ACAUACAGGAUUGUG	ID. NO. 84
386	AGGAAAACCCACCUUA CAUACAGGAUUGUGA	ID. NO. 85
390	AAACCCACCUUACAU CAGGAUUGUGAAUUA	ID. NO. 86
397	CCUACAUACAGGAUU GUGAAUUAUACACCA	ID. NO. 87
404	UACAGGAUUGUGAAUU AUACACCAGAUUUGC	ID. NO. 88
405	ACAGGAUUGUGAAUUA UACACCAGAUUUGCC	ID. NO. 89
407	AGGAUUGUGAAUUA CACCAGAUUUGCCAA	ID. NO. 90
416	AUUUAUACACCAGAUU UGCCAAAAGAUUGCUG	ID. NO. 91
417	AUUUAUACACCAGAUU GCCAAAAGAUUGCUGU	ID. NO. 92
433	GCCAAAAGAUUGCUGU GAUUCUGCUGUUGAG	ID. NO. 93
437	AAAGAUUGCUGUUGAUU CUGCUGUUGAGAAAG	ID. NO. 94
438	AAGAUUGCUGUUGAUUC UGCUGUUGAGAAAGC	ID. NO. 95
445	UGUUGAUUCUGCUGU GAGAAAGCUCUGAAA	ID. NO. 96

455	GCUGUUGAGAAAGCUC UGAAAGUCUGGGAAG	ID. NO. 97
463	GAAAGCUCUGAAAGUC UGGAAGAGGUGACU	ID. NO. 98
479	UGGAAGAGGUGACUC CACUCACAUUCUCCA	ID. NO. 99
484	AGAGGUGACUCCACUC ACAUUCUCCAGGCUG	ID. NO. 100
489	UGACUCCACUCACAUU CUCCAGGCUGUAUGA	ID. NO. 101
490	GACUCCACUCACAUUC UCCAGGCUGUAUGAA	ID. NO. 102
492	CUCCACUCACAUUCUC CAGGCUGUAUGAAGG	ID. NO. 103
501	CADUCUCCAGGCUGUA UGAAGGAGAGGCUGA	ID. NO. 104
518	GAAGGAGAGGCUGAUA UAAUGAUCUCUUUG	ID. NO. 105
520	AGGAGAGGCUGAUAUA AUGAUCUCUUUGCA	ID. NO. 106
526	GGCUGAUAUAUGAUC UCUUUGCAGUAGA	ID. NO. 107
528	CUGAUAUAUGAUCUC UUUUGCAGUAGAGA	ID. NO. 108
530	GAUAUAUGAUCUCUU UUGCAGUAGAGAAC	ID. NO. 109
531	AAUAUAUGAUCUCUUU UGCAGUAGAGAAC	ID. NO. 110
532	UAUAUAUGAUCUCUUU GCAGUAGAGAACAU	ID. NO. 111
538	GADCUCUUUUGCAGUU AGAGAACAUUGGAGAC	ID. NO. 112
539	AUCUCUUUUGCAGUUA GAGAACAUUGGAGACU	ID. NO. 113
555	GAGAACAUUGGAGACUU UUAACCCUUUGAUGG	ID. NO. 114
556	AGAACAUGGAGACUUU UAACCCUUUGAUGGA	ID. NO. 115
557	GAACAUGGAGACUUU AACCCUUUGAUGGAC	ID. NO. 116
558	AACAUGGAGACUUUA OCCUUUGAUGGACC	ID. NO. 117
563	GGAGACUUUAACCCUU UUGAUGGACCUGGAA	ID. NO. 118
564	GAGACUUUAACCCUUU UGAUGGACCUGGAAA	ID. NO. 119
565	AGACUUUAACCCUUU GAUGGACCUGGAAAU	ID. NO. 120
583	UGGACCUGGAAAUUUU UUGGCCAUGCCUAU	ID. NO. 121
584	GGACCUGGAAAUUUU UGGGCCAUGCCUAUG	ID. NO. 122
585	GACCUGGAAAUUUU GGGCCAUGCCUAUGC	ID. NO. 123
597	UUUUGGCCAUGCCUA UGGGCCUGGGCCAGG	ID. NO. 124
616	CCUGGGCCAGGGAUU AAUGGAGAUGCCAC	ID. NO. 125
617	CCUGGGCCAGGGAUUA AUGGAGAUGCCACU	ID. NO. 126
633	AUGGAGAUGCCACUU UGAUGAUGAUGAACA	ID. NO. 127
634	UGGAGAUGCCACUUU GAUGAUGAUGAACA	ID. NO. 128
662	CAUGGACAAAGGUA CAACAGGGACCAAUU	ID. NO. 129
677	ACAACAGGGACCAAUU UAUUUCUGUUGCUG	ID. NO. 130
678	CAACAGGGACCAAUU AUUUCUGUUGCUGC	ID. NO. 131
679	AACAGGGACCAAUUA UUUUCUGUUGCUGCU	ID. NO. 132

681	CAGGGACCAUUUUAU	UCUUGUUGCUCUCA	ID. NO. 133
682	AGGGACCAUUUUAU	CUUGUUGCUCUCAU	ID. NO. 134
683	GGGACCAUUUUAUUC	UCGUGCUGGCUCAUG	ID. NO. 135
685	GACCAUUUUAUUCUC	GUUGCUGCUC AUGAA	ID. NO. 136
688	CAUUUAUUUCUGUU	GCUGCUC AUGAAAU	ID. NO. 137
695	UUUCUGUUGCUCUC	AUGAAAUUGGOCACU	ID. NO. 138
703	UGCUGCUC AUGAAAU	GGOCACUCCUGGGU	ID. NO. 139
711	AUGAAAUUGGOCACUC	CCUGGGUCUCUUUCA	ID. NO. 140
719	GGOCACUCCUGGGUC	UCUUUCACUCAGCCA	ID. NO. 141
721	CCACUCCUGGGUCUC	UUUCACUCAGCCAAC	ID. NO. 142
723	ACUCCUGGGUCUCUU	UCACUCAGCCAACAC	ID. NO. 143
724	CUCCUGGGUCUCUU	CACUCAGCCAACACU	ID. NO. 144
725	UCCUGGGUCUCUUUC	ACUCAGCCAACACUG	ID. NO. 145
729	UGGGUCUCUUUCACUC	AGCCAACACUGAAGC	ID. NO. 146
746	GCCAACACUGAAGCUU	UGAUGUACCCACUCU	ID. NO. 147
747	CCAACACUGAAGCUUU	GAUGUACCCACUCUA	ID. NO. 148
753	CUGAAGCUUUGAUGUA	CCCACUCUAUCACUC	ID. NO. 149
760	UUUGAUGUACCCACUC	UAUCACUCACUCACA	ID. NO. 150
762	UGAUGUACCCACUCUA	UCACUCACUCACAGA	ID. NO. 151
764	AUGUACCCACUCUAUC	ACUCACUCACAGACC	ID. NO. 152
768	ACCCACUCUAUCACUC	ACUCACAGACCUGAC	ID. NO. 153
772	ACUCUAUCACUCACUC	ACAGACCUGACUUGG	ID. NO. 154
785	CUCACAGACCUGACUC	GGUCCGOCUGUCUC	ID. NO. 155
789	CAGACCUGACUUGGUU	CGCCUGUCUCAAGA	ID. NO. 156
790	AGACCUGACUUGGUUC	CGCCUGUCUCAAGAU	ID. NO. 157
798	CUCGGUCCGOCUGUC	UCAAGAUUAUUAUA	ID. NO. 158
800	CGGUCCGOCUGUCUC	AAGAUUAUUAUAUG	ID. NO. 159
809	CUGUCUCAAGAUUAUA	UAAUUGGCAUUCAGU	ID. NO. 160
811	GUCUCAAGAUUAUAUA	AAUUGGCAUUCAGUCC	ID. NO. 161
820	UGAUUAUAUAUUGGCAU	CAGUCCUUAUGGA	ID. NO. 162
821	GAUAUAUAUAUUGGCAUUC	AGUCCUUAUGGAC	ID. NO. 163
825	UAAUUGGCAUUCAGUC	CCUUAUGGACCUCC	ID. NO. 164
829	UGGCAUUCAGUCCUCC	UAUGGACCUCCUCCU	ID. NO. 165
831	GCAUUCAGUCCUUAUA	UGGACCUCCUCCUGA	ID. NO. 166
839	UCCUUAUGGACCUCC	CCUUAUGGACCUCCUG	ID. NO. 167
849	GACCUCCUCCUGACUC	CCUUAUGGACCUCCU	ID. NO. 168

868	UGAGACCCCCUUGUA CCCACGGAAACCUGUC	ID. NO. 169
883	ACCCACGGAAACCUGUC CCUCCAGAACCUGGG	ID. NO. 170
887	AACGAAACCUGUCCUUC CAGAACCCUGGACGC	ID. NO. 171
917	CCAGCCAACUGUGAUC CUGCUUUGUCCUUUG	ID. NO. 172
923	AACUGUGAUCCUGCUU UGUCCUUUGAUGCUG	ID. NO. 173
924	ACUGUGAUCCUGCUU GUCCUUUGAUGCUGU	ID. NO. 174
927	GUGAUCCUGCUUUGUC CUUGAUGCUGUCAG	ID. NO. 175
930	AUCCUGCUUUGUCCUU UGAUGCUGUCAGCAC	ID. NO. 176
931	UCCUGCUUUGUCCUU GAUGCUGUCAGCACU	ID. NO. 177
940	GUCCUUUGAUGCUGUC AGCACUCUGAGGGGA	ID. NO. 178
947	GAUGCUGUCAGCACUC UGAGGGGAGAAUCC	ID. NO. 179
961	UCUGAGGGGAGAAUUC CUGAUUUUAAAGAC	ID. NO. 180
967	GGGAGAAUCCUGAUC UUAAGACAGGCAC	ID. NO. 181
969	GAGAAUCCUGAUUUU UAAAGACAGGCACUU	ID. NO. 182
970	AGAAAUCCUGAUUUU AAAGACAGGCACUUU	ID. NO. 183
971	GAAAUCCUGAUUUU AAAGACAGGCACUUU	ID. NO. 184
984	UUAAGACAGGCACUU UGGGCGAAAUCCUC	ID. NO. 185
985	UAAAGACAGGCACUUU UGGGCGAAAUCCUC	ID. NO. 186
986	AAAGACAGGCACUUU GGGCGAAAUCCUCA	ID. NO. 187
996	ACUUUUGGCGAAAUCC CCUCAGTAAGCUUGA	ID. NO. 188
1000	UUGGCGCGAAAUCCUC AGGAAGCUUGAACCU	ID. NO. 189
1009	AUCCUCAGGAAGCUU GAACCUGAUUGCAU	ID. NO. 190
1020	AGCUUGAACCUGAUU GCAUUGAUCUCUUC	ID. NO. 191
1025	GAACCUGAUUGCAUU UGAUCUCUUCAUUU	ID. NO. 192
1026	AACCUGAUUGCAUUU GAUCUCUUCAUUUUG	ID. NO. 193
1030	UGAAUUGCAUUGAUC UCUUCAUUUUGGCGA	ID. NO. 194
1032	AAUUGCAUUGAUCUC UUCAUUUUUGGCCAUC	ID. NO. 195
1034	UUGCAUUGAUCUCU CAUUUUUGGCCAUCUC	ID. NO. 196
1035	UGCAUUGAUCUCUUC AUUUUGGCCAUCUCU	ID. NO. 197
1038	AUUUGAUCUCUUCAUU UUGGCCAUCUCUUC	ID. NO. 198
1039	UUUGAUCUCUUCAUUU UGGCCAUCUCUUCU	ID. NO. 199
1040	UGAUCUCUUCAUUUU GGCCAUCUCUUCUU	ID. NO. 200
1047	CUUCAUUUUGGCCAUC UCUUCCUUCAGGGU	ID. NO. 201
1049	UCAUUUUUGGCCAUCUC UUCUUCAGGGUGG	ID. NO. 202
1051	AUUUUUGGCCAUCUCU CCUUCAGGGUGGAU	ID. NO. 203
1052	UUUUUGGCCAUCUCUUC CUUCAGGGUGGAUG	ID. NO. 204

1055	UGGOCACUCUUCUUU CAGGOGUGGALUGOOG	ID. NO. 205
1056	GGCCACUCUCUUCUUC AGGOGUGGALUGOOGC	ID. NO. 206
1074	GOGUGGALUGOOGCAUA UGAAGUUAUAGCAA	ID. NO. 207
1081	UGOOGCAUAUGAAGUU ACUAGCAAGGACCUC	ID. NO. 208
1082	GCOGCAUAUGAAGUUA CUAGCAAGGACCUCG	ID. NO. 209
1085	GCAUAUGAAGUUAUA GCAAGGACCUCGUUU	ID. NO. 210
1096	UAUAAGCAAGGACCUC GUUUUUAUUUAAA	ID. NO. 211
1099	UAGCAAGGACCUCGUU UUCAUUUUUAAAGGA	ID. NO. 212
1100	AGCAAGGACCUCGUUU UCAUUUUUAAAGGAA	ID. NO. 213
1101	GCAAGGACCUCGUUUU CAUUUUUAAAGGAAA	ID. NO. 214
1102	CAAGGACCUCGUUUUC AUUUUUAAAGGAAU	ID. NO. 215
1105	GGACCUCGUUUUCAU UUUAAAGGAAAUCAA	ID. NO. 216
1106	GACCUCGUUUUCAUU UUAAGGAAAUCAAU	ID. NO. 217
1107	ACCUCGUUUUCAUUU UAAAGGAAAUCAAU	ID. NO. 218
1108	CCUCGUUUUCAUUUU AAAGGAAAUCAAUUC	ID. NO. 219
1109	CUUGUUUCAUUUUUA AAGGAAAUCAAUUCU	ID. NO. 220
1118	AUUUUUAAAGGAAUC AAUUCUGGGOCAUCA	ID. NO. 221
1122	UUAAGGAAAUCAAUU CUGGGCCAUCAGAGG	ID. NO. 222
1123	UAAAGGAAAUCAAUUC UGGGCCAUCAGAGGA	ID. NO. 223
1132	UCAAUUCUGGGCCAUC AGAGGAAUAGAGGUA	ID. NO. 224
1147	CAGAGGAAUAGAGGUA CGAGCUGGALUACCA	ID. NO. 225
1158	AGGUACGAGCUGGUAU CCAAGAGGCAUCCA	ID. NO. 226
1171	AUAACCAAGAGGCAUC CACACCCUAGGUUUC	ID. NO. 227
1180	AGGCAUCCACACCUUA GGUUUCCUCCAACC	ID. NO. 228
1184	AUCCACACCCUAGGUU UCCCUCCAACCGUGA	ID. NO. 229
1185	UCCACACCCUAGGUU CCCCUCACCGUGAG	ID. NO. 230
1186	CCACACCCUAGGUUUC CCUCACACCGUGAGG	ID. NO. 231
1190	ACCCUAGGUUUCUUC CAACCGUGAGGAAAA	ID. NO. 232
1207	AACCGUGAGGAAAAUC GAUGCAGCCAUUUCU	ID. NO. 233
1219	AAUGAUGCAGCCAUU UCUGAUUAGGAAAAG	ID. NO. 234
1220	AUUGAUGCAGCCAUU CUGAUUAGGAAAAGA	ID. NO. 235
1221	UUGAUGCAGCCAUUC UGAUAAGGAAAAGAA	ID. NO. 236
1226	GCAGCCAUUUCUGAUA AGGAAAAGAACAAAA	ID. NO. 237
1245	AAAAGAACAAAACAU UUUUUUGUAGAGGA	ID. NO. 238
1247	AGAACAACAAACAUU UCUUUGUAGAGGACA	ID. NO. 239
1248	AGAACAACAAACAUUU CUUUGUAGAGGACAA	ID. NO. 240

1249	GAACAAAACAUUUUC UUUGUAGAGGACAAA	ID. NO. 241
1251	ACAAAACAUUUUCUU UGUAGAGGACAAAU	ID. NO. 242
1252	CAAAAACAUUUUCUU GUAGAGGACAAAUAC	ID. NO. 243
1255	AACAUUUUCUUUGUA GAGGACAAAUACUGG	ID. NO. 244
1266	UUUGUAGAGGACAAAU CUGGAGAUUUGAUGA	ID. NO. 245
1275	ACAAAUACUGGAGAUU UGAUGAGAAGAGAAA	ID. NO. 246
1276	CAAAUACUGGAGAUU GAUGAGAAGAGAAU	ID. NO. 247
1292	GAUGAGAAGAGAAAU CC AUGGAGGCCAGGCU	ID. NO. 248
1293	AUGAGAAGAGAAAUUC CAUGGAGGCCAGGCU	ID. NO. 249
1308	CC AUGGAGGCCAGGCUU UCCCAAGCAAUAGC	ID. NO. 250
1309	CAUGGAGGCCAGGCUU CC CAAGCAAUAGCU	ID. NO. 251
1310	AUGGAGGCCAGGCUUC CC AAGCAAUAGCUG	ID. NO. 252
1321	CUUUCCCAAGCAAUA GCUGAAGACUUUCCA	ID. NO. 253
1332	AAAUAGCUGAAGACUU UCCAGGGAUUGACUC	ID. NO. 254
1333	AAUAGCUGAAGACUU CCAGGGAUUGACUCA	ID. NO. 255
1334	AUAGCUGAAGACUUUC CAGGGAUUGACUCA	ID. NO. 256
1342	AGACUUCCAGGGAUU GACUCAAGAUUGAU	ID. NO. 257
1347	UCCAGGGAUUGACUC AAAGAUUGAUGCUGU	ID. NO. 258
1354	GAUUGACUCAAGAUU GAUGCUGUUUUGAA	ID. NO. 259
1363	AAAGAUUGAUGCUGU UUUGAAGAUUUGGG	ID. NO. 260
1364	AAGAUUGAUGCUGUU UGAAGAUUUGGGU	ID. NO. 261
1365	AGAUUGAUGCUGUUU UGAAGAUUUGGGU	ID. NO. 262
1366	GAUUGAUGCUGUUUU GAAGAUUUGGGUUC	ID. NO. 263
1374	CUGUUUUGAAGAUU UGGGUUCUUUUAUU	ID. NO. 264
1375	UGUUUUGAAGAUU GGGUUCUUUUAUUC	ID. NO. 265
1380	UGAAGAUUUGGGU CUUUUAUUUCUUAC	ID. NO. 266
1381	UGAAGAUUUGGGUUC UUUUAUUUCUUACU	ID. NO. 267
1383	AAGAUUUGGGUUCUU UUAUUUCUUACUGG	ID. NO. 268
1384	AGAUUUGGGUUCUU UAUUCUUACUGGA	ID. NO. 269
1385	GAAUUGGGUUCUUU AUUCUUACUGGAU	ID. NO. 270
1386	AAUUGGGUUCUUUA UUUCUUACUGGAUC	ID. NO. 271
1388	UUUGGGUUCUUUAU UCUUUACUGGAUCU	ID. NO. 272
1389	UUGGGUUCUUUAUU CUUACUGGAUCUUC	ID. NO. 273
1390	UGGGUUCUUUAUUC UUUACUGGAUCUCA	ID. NO. 274
1392	GGUUCUUUAUUCUU UACUGGAUCUUCACA	ID. NO. 275
1393	GUUCUUUAUUCUU ACUGGAUCUUCACAG	ID. NO. 276

1394	UUCUUUUAUUUCUUA	CUGGAUCUUCACAGU	ID. NO. 277
1401	AUUUCUUUACUGGAUC	UUCACAGUUGGAGUU	ID. NO. 278
1403	UUCUUUACUGGAUCUU	CACAGUUGGAGUUUG	ID. NO. 279
1404	UCUUUACUGGAUCUUC	ACAGUUGGAGUUUGA	ID. NO. 280
1410	CUGGAUCUUCACAGUU	GGAGUUUGACCCAAA	ID. NO. 281
1416	CUUCACAGUUGGAGUU	UGACCCAAAUGCAAA	ID. NO. 282
1417	UUCACAGUUGGAGUUU	GACCCAAAUGCAAAG	ID. NO. 283
1448	AAAGUGACACACACUU	UGAAGAGUACAGCU	ID. NO. 284
1449	AAGUGACACACACUUU	GAAGAGUACAGCUG	ID. NO. 285
1457	CACACUUUGAAGAGUA	ACAGCUGGCUUAAUU	ID. NO. 286
1468	GAGUACAGCUGGCUU	AAUUGUGAAAGAGA	ID. NO. 287
1469	AGUACAGCUGGCUUA	AUUGUUGAAAGAGAU	ID. NO. 288
1472	AACAGCUGGCUUAAUU	GUUGAAAGAGAUUG	ID. NO. 289
1475	AGCUGGCUUAAUUGUU	GAAAGAGAUUUGUAG	ID. NO. 290
1485	AUUGUUGAAAGAGAU	UGUAGAAGGCACAAU	ID. NO. 291
1489	UUGAAAGAGAUUUGUA	GAAGGCACAAUUGG	ID. NO. 292
1501	UGUAGAAGGCACAUA	UGGGCACUUUAAUUG	ID. NO. 293
1510	CACAUAUUGGGCACUU	UAAUGAAGCUAAUA	ID. NO. 294
1511	ACAUAUUGGGCACUUU	AAAUGAAGCUAAUA	ID. NO. 295
1512	CAUAUUGGGCACUUUA	AAUGAAGCUAAUAU	ID. NO. 296
1522	ACUUUAAUGAAGCUA	AUAUUCUUCACCUA	ID. NO. 297
1525	UUAAUUGAAGCUAUA	AUUCUUCACCUAAGU	ID. NO. 298
1528	AAUGAAGCUAAUAUU	CUUCACCUAAGUCUC	ID. NO. 299
1529	AUGAAGCUAAUAUUC	UUCACCUAAGUCUCU	ID. NO. 300
1531	GAAGCUAAUAUUCUU	CACCUAAGUCUCUGU	ID. NO. 301
1532	AAGCUAUAUUCUUC	ACCUAAGUCUCUGUG	ID. NO. 302
1537	AUAUUCUUCACCUA	AGUCUCUGUGAAUUG	ID. NO. 303
1541	AUUCUUCACCUAAGUC	UCUGUGAAUUGAAAU	ID. NO. 304
1543	UCUUCACCUAAGUCUC	UGUGAAUUGAAUUGU	ID. NO. 305
1551	UAAGUCUCUGUGAAU	GAAUUGUUGUUUUC	ID. NO. 306
1559	UGUGAAUUGAAUUGU	UGUUUUCUCCUGCCU	ID. NO. 307
1560	GUGAAUUGAAUUGUUC	GUUUUUCUCCUGCCUG	ID. NO. 308
1563	AAUUGAAUUGUUGUU	UUCUCCUGCCUGGUC	ID. NO. 309
1564	AUUGAAUUGUUGUUU	UCUCCUGCCUGGUCU	ID. NO. 310
1565	UUGAAUUGUUGUUUU	CUCCUGCCUGGUGUG	ID. NO. 311
1566	UGAAUUGUUGUUUUC	UCCUGCCUGGUGUGU	ID. NO. 312

1568	AAALGUUCGUUUUCUC CUGCCUGUGCUGUGA	ID. NO. 313
1586	GCCUGUGCUGUGACUC GAGUCACACUCAAGG	ID. NO. 314
1591	UGCUGUGACUCGAGUC ACACUCAAGGGAACU	ID. NO. 315
1597	GACUOGAGUCACACUC AAGGGAACUUGAGCG	ID. NO. 316
1607	ACACUCAAGGGAACU GAGCGUGAUCUGUA	ID. NO. 317
1618	AACUUGAGCGUGAUC UGUUUCUUGCCGGUC	ID. NO. 318
1622	UGAGCGUGAUCUGUA UCUUGCCGGUCAUUU	ID. NO. 319
1624	AGCGUGAUCUGUAUC UUGCCGGUCAUUUU	ID. NO. 320
1626	CGUGAUCUGUAUCU GCGGGUCAUUUUUAU	ID. NO. 321
1633	CUGUAUCUUGCCGGUC AUUUUUAUGUAUUA	ID. NO. 322
1636	UAUCUUGCCGGUCAU UUUAUGUAUUAACAG	ID. NO. 323
1637	AUCUUGCCGGUCAUU UAUGUAUUAACAGG	ID. NO. 324
1638	UCUUGCCGGUCAUUU UAUGUAUUAACAGG	ID. NO. 325
1639	CUUGCCGGUCAUUUU AUGUAUUAACAGGC	ID. NO. 326
1640	UUGCCGGUCAUUUUA UGUUAUUAACAGGCA	ID. NO. 327
1644	CGGUCAUUUUUAUGU AUUAACAGGGCAUUA	ID. NO. 328
1645	GGUCAUUUUUAUGUA UAACAGGGCAUUA	ID. NO. 329
1647	UCAUUUUUAUGUAUUA ACAGGGCAUUAUUA	ID. NO. 330
1648	CAUUUUUAUGUAUUA CAGGGCAUUAUUA	ID. NO. 331
1657	GUUAUUAACAGGGCAU CAAUUGGGCUGCUGC	ID. NO. 332
1658	UAUAUUAACAGGGCAU AAUUGGGCUGCUGCU	ID. NO. 333
1674	AAUUGGGCUGCUGCUU AGCUUGCACCUUGUC	ID. NO. 334
1675	AAUUGGGCUGCUGCUA GCUUGCACCUUGUCA	ID. NO. 335
1679	GGCUGCUGCUUAGCUU GCACCUUGUCACAU	ID. NO. 336
1686	GCUUAGCUUGCACCU GUCACAUAGAGUGAU	ID. NO. 337
1689	UAGCUUGCACCUUGUC ACAUAGAGUGAUCU	ID. NO. 338
1694	UGCACCUUGUCACAU GAGUGAUCUUUCCCA	ID. NO. 339
1702	GUCACAUAGAGUGAUC UUUCCCAAGAGAAGG	ID. NO. 340
1704	CACAUAGAGUGAUCU UCCCAAGAGAAGGGG	ID. NO. 341
1705	ACAUAGAGUGAUCUUU CCCAAGAGAAGGGGA	ID. NO. 342
1706	CAUAGAGUGAUCUUU CCAAGAGAAGGGGAA	ID. NO. 343
1727	AGAAGGGGAAGCACUC GUGUGCAACAGACAA	ID. NO. 344
1751	CAGACAAGUGACUGUA UCUUGUGUAGACUAU	ID. NO. 345
1753	GACAAGUGACUGUAUC UGUGUAGACUAUUUG	ID. NO. 346
1759	UGACUGUAUCUGUGA GACUAUUUGCUUAU	ID. NO. 347
1764	GUUUCUGUGUAGACUA UUUGCUUAUUUAUA	ID. NO. 348

1766	AUCUGUGUAGACUAAU UGCUAAUUAUAUAA	ID. NO. 349
1767	UCUGUGUAGACUAAU GCUAAUUAUAUAAAG	ID. NO. 350
1771	UGUAGACUAAUUGCUU AUUAUAUAAGACGA	ID. NO. 351
1772	GUAGACUAAUUGCUUA UUAUAUAAGACGAU	ID. NO. 352
1774	AGACUAAUUGCUAAU UAUAUAAGACGAUU	ID. NO. 353
1775	GACUAAUUGCUAAUU AAUAUAAGACGAUUG	ID. NO. 354
1776	ACUAAUUGCUAAUUA AUUAUAAGACGAUUGU	ID. NO. 355
1779	AUUUGCUAAUUAUA AAGACGAUUGUCAG	ID. NO. 356
1788	UUAUAUAAGACGAU UGUCAGUUGUUU	ID. NO. 357
1789	UUAUAUAAGACGAUU GUCAGUUGUUU	ID. NO. 358
1792	AUAUAAGACGAUUGUC AGUUGUUU	ID. NO. 359

Table AIV: Human Stromelysin HP Target Sequence

nt. Position	Target Sequence	Seq. ID. NO.
66	CUACU GUU GCUGUGGUGGCAGU	ID. NO. 360
82	UGGCA GUU UGCUCAGCCUAUCCA	ID. NO. 361
192	AAACA GUU UGUUAGGAGAAAGGA	ID. NO. 362
430	AUGCU GUU GAUUCUGCUGUUGAG	ID. NO. 363
442	CUGCU GUU GAGAAAGCUCUGAAA	ID. NO. 364
775	UCACA GAC CUGACUUGGUUCCGC	ID. NO. 365
1360	AUGCU GUU UUUGAAGAAUUUGGG	ID. NO. 366
1407	UCACA GUU GGAGUUUGACCCAAA	ID. NO. 367

Table AV: Human HH Ribozyme Sequence

nt. Position.	Ribozyme Sequence	Seq. ID.
10	GUUGUCUC CUGAAGAGCACGAAAGUGCGAA AUGCCUUG	ID.NO.375
21	UUAGCUC CUGAUGAGGCCGAAAGGCCGAA AUGUUGU	ID.NO.376
168	GAGGUUG CUGAUGAGGCCGAAAGGCCGAA AGUAGUU	ID.NO.377
616	CUCCAUU CUGAUGAGGCCGAAAGGCCGAA AUCCCTUG	ID.NO.378
617	UCUCCAU CUGAUGAGGCCGAAAGGCCGAA AAUCCCU	ID.NO.379
633	CAUCAUCA CUGAAGAGCACGAAAGUGCGAA AGUGGGCA	ID.NO.380
634	UCAUCAUC CUGAAGAGCACGAAAGUGCGAA AAGUGGGC	ID.NO.381
662	CCUGUUG CUGAUGAGGCCGAAAGGCCGAA AUCCUUU	ID.NO.382
711	ACCCAGG CUGAUGAGGCCGAAAGGCCGAA AGUGGCC	ID.NO.383
820	GGGACUG CUGAUGAGGCCGAAAGGCCGAA AUGCCAU	ID.NO.384
883	UCUGGAGG CUGAAGAGCACGAAAGUGCGAA ACAGGUUC	ID.NO.385
947	CCCCUCA CUGAUGAGGCCGAAAGGCCGAA AGUGCUG	ID.NO.386
996	CCUGAGG CUGAUGAGGCCGAAAGGCCGAA AUUUGCG	ID.NO.387
1123	UGGCCCA CUGAUGAGGCCGAAAGGCCGAA AAUUGAU	ID.NO.388
1132	UUUCCUCU CUGAUGAGCACGAAAGUGCGAA AUGGCCCA	ID.NO.389
1221	CCUUUCA CUGAAGAGCACGAAAGUGCGAA AAAUGGCU	ID.NO.390
1266	UCUCCAG CUGAUGAGGCCGAAAGGCCGAA AUUUGUC	ID.NO.391
1275	UCUCAUCA CUGAAGAGCACGAAAGUGCGAA AUCUCCAG	ID.NO.392
1334	AUCCCTUG CUGAUGAGGCCGAAAGGCCGAA AAAGUCU	ID.NO.393
1354	CAGCAUC CUGAUGAGGCCGAAAGGCCGAA AUCUUUG	ID.NO.394
1363	UCTUCAAA CUGAUGAGCACGAAAGUGCGAA ACAGCAUC	ID.NO.395
1410	AAACUCC CUGAUGAGGCCGAAAGGCCGAA ACUGUGA	ID.NO.396

Table AVI: Rabbit Stromelysin HH Ribozyme Target Sequence

nt. Position	Target Sequence	nt. Position	Target Sequence
18	CAAGGCAU C AAGACAGC	345	CCUGAUGU U GGUCACUU
29	GACAGCAU A GAGCUGAG	349	AUGUUGGU C ACUUCAGU
39	AGCUGAGU A AAGCCAAU	353	UGGUCACU U CAGUACCU
61	UGAAAACU C UUCCAACC	354	GGUCACUU C AGUACCUU
63	AAAACUCU U CCAACCCU	358	ACUUCAGU A CCUUCUUU
64	AAACUCUU C CAACCCUG	362	CAGUACCU U CCCUGGCA
75	ACCCUGCU A CUGCUGUG	363	AGUACCUU C CCUGGCAC
93	GUGGCGCU U UGCUCAGC	391	CAAAAACU C ACCUAACT
94	UGGCGCUU U GCUCAGCC	396	ACUCACCU A ACUAGGAG
98	GCUUUGCU C AGCCUAUC	400	ACCUAACT U ACAGGAUU
104	CUCAGCCU A UCCACUGG	401	CCUAACTU A CAGGAUUG
106	CAGCCUAA C CACUGGAU	408	UACAGGAU U GUGAAUUA
122	UGGAGCCU C AAGGGAUG	415	UUGUGAAU U ACACACCG
153	AUGGACCU U CUUCAGCA	416	UGUGAAUU A CACACCGG
154	UGGACCUU C UUCAGCAA	427	CACCGGAU C UGCCAAGA
156	GACCUUCU U CAGCAAUU	444	GAUGCUGU U GAUGCUGC
157	ACCUUCUU C AGCAAUAU	456	GCUGCCAU U GAGAAAGC
164	UCAGCAAU A UCUGGAAA	466	AGAAAGCU C UGAAGGUC
166	AGCAAUAU C UGGAAAAC	474	CUGAAGGU C UGGGAGGA
176	GGAAAACU A CUACAACC	490	AGGUGACT C CACUCACG
179	AAACUACU A CAACCUUG	495	ACUCCACT C ACGUUCUC
186	UACAACCU U GAAAAAGA	500	ACUCACGU U CUCCAGGA
206	GAAACAGU U UGUAAAAA	501	CUACAGUU C UCCAGGAA
207	AAACAGUU U GUUAAAAG	503	CACGUUCU C CAGGAAGU
210	CAGUUGU U AAAAGAAA	512	CAGGAAGU A UGAAGGAG
211	AGUUGUU A AAAGAAAG	531	GCUGACAU A AUGAUCUC
226	AGGACAGU A GUCCUGUU	537	AUAUUGAU C UCUUUUGG
229	ACAGUAGU C CUGUUGUU	539	AAUGAUCU C UUUUGGAG
234	AGUCCUGU U GUUAAAAA	541	UGAUCUCU U UUGGAGUC
237	CCUGUUGU U AAAAAAAU	542	GAUCUCUU U UGGAGUCC
238	CUGUUGUU A AAAAAAUC	543	AUCUCUUU U GGAGUCCG
246	AAAAAAAU C CAAGAAAU	549	UUUGGAGU C CGAGAACA
263	GCAGAAGU U CCUUGGCU	565	AUGGAGAU U UUAUUCCU
264	CAGAAGUU C CUUGGCUU	566	UGGAGAUU U UAUUCCUU
267	AAGUUCU U GGCUGGA	567	GGAGAUUU U AUUCCUUU
272	CCUUGGCU U GGAGGUGA	568	GAGAUUUU A UUCCUUUU
296	GCUGGACU C CAACACCC	570	GAUUUUUU U CCUUUUGA
315	GAGGUGAU A CGCAAGCC	571	AUUUUUUU C CUUUUGAU
336	UGUGGCGU U CCUGAUGU	574	UUUUUUUU U UUGAUGGA
337	GUGGCGUU C CUGAUGUU	575	UAUUUUUU U UGAUGGAC

576	AUUCUUU U GAUGGACC	905	UCCAGGAU C UGGGACCC
594	GGAAAUGU U UUGGCUCA	918	ACCCAGU C AUGUGUGA
595	GAAAUGUU U UGGCUCAU	928	UGUGUGAU C CAGAUUCG
596	AAAUGUUU U GGCUCAUG	934	AUCCAGAU C UGUCCUUC
601	UUUUGGCU C AUGCUUUA	938	AGAUUCUG C CUUCGAUG
607	CUCAUGCU U AUGCACCU	941	UCUGUCCU U CGAUGCAA
608	UCAUGCUU A UGCACCUG	942	CUGUCCUU C GAUGCAAU
627	CCAGGAU U AAUGGAGA	951	GAUGCAAU C AGCACUCU
628	CAGGAUU A AUGGAGAU	958	UCAGCACU C UGAGGGGA
644	UGCCCAU U UGAUGAUG	972	GGAGAAU U CUGUUCUU
645	GCCCAU U GAUGAUGA	973	GAGAAAU C UGUUCUUU
673	CAAAGGAU A CAACAGGA	977	AAUUCUGU U CUUAAAG
688	GAACCAU U UAUCCUU	978	AUUCUGU C UUUAAAGA
689	AACCAAU U AUUCUUG	980	UCUGUUCU U UAAAGACA
690	ACCAAUU A UUCUUGU	981	CUGUUCUU U AAAGACAG
692	CAAUUUU U CCUGUUG	982	UGUUCUUU A AAGACAGG
693	AAUUUAU C CUUGUUGC	992	AGACAGGU A UUUCUGGC
696	UUAUUCU U GUUGCUGC	994	ACAGGAU U UCUGGCGC
699	UUCUUGU U GCUGCUCA	995	CAGGAUU U CUGGCGCA
706	UUGCUGU C AUGAGCUU	996	AGGAUUU C UGGCGCAA
714	CAUGAGCU U GGCCACUC	1007	GCGCAUU C CCUCAGGA
722	UGGCCAU C CCUGGGUC	1011	AAGUCCU C AGGAUUCU
730	CCUUGGU C UGUUUCAC	1017	CUAGGAU U CUCGAACC
734	GGGUCUGU U UCAUCGG	1018	UCAGGAU C UCGAACCU
735	GGUCUGU U CACUCGGC	1020	AGGAUUCU C GAACCTUGA
736	GUCUGUU C ACUCGGCC	1031	ACCUGAGU U UCAUUUGA
74	GUUUCACU C GGCCAACC	1032	CCUGAGUU U CAUUUGAU
75	GCUGADGU A CCCAGUCU	1033	CUGAGUUU C AUUUGAUC
771	UACCCAGU C UACAACGC	1036	AGUUUCAU U UGAUCUCU
773	CCCAGUCU A CAACGCCU	1037	GUUUCAU U GAUCUCUU
782	CAACGCCU U CACAGACC	1041	CAUUUGAU C UCUCAUU
783	AACGCCU C ACAGACCU	1043	UUUGAUU C UUCAUUCU
800	GGCCCGU U CCGCCUUU	1045	UGAUCUCU U CAUUCUGG
801	GCCCGGU C CGCCUUUC	1046	GAUCUCU C AUUCUGGC
807	UUCCGCCU U UCUCAAGA	1049	CUCUUCU C UUGGCCAU
808	UCCGCCU U CUCAAGAU	1050	UCUUCAU C UGGCCAUC
809	CCGCCUU C UCAAGAUG	1058	CUGGCCAU C UCUCUUU
811	GCCUUUCU C AAGAUGAU	1060	GGCCAUCU C UUCUUUCA
831	GAUGGCAU C CAAUCCU	1062	CCAUUCU U CCUCAGC
836	CAUCCAAU C CCUCUAUG	1063	CAUCUCU C CUUCAGCA
840	CAAUCCU C UAUGGACC	1066	CUUUCU U CAGCAGUG
842	AUCCUCU A UGGACCGG	1067	UCUCCU C AGCAGUGG
860	CCUGCCU C UCCUGAU	1085	UGCUGAU A UGAAGUUA
862	CUGCCUCU C CUGAUAA	1092	UAUGAGU U AUUAGCAG
868	CUCCUGAU A ACUCUGGA	1093	AUGAAGU A UUAGCAGG
872	UGAUAAU C UGGAGUGC	1095	GAGUUU U AGCAGGGA
883	GAGUGCCU A UGGAACCU	1096	AAGUUU A GCAGGGAU
894	GAACUCU C CCUCCAGG	1105	GCAGGGAU A CUGUUUUC
898	CUGUCCU C CAGGAUCU	1110	GAGUCU U UUCAUUU

1111	AUACUGUU U UCAUUUUU	1374	GAUGCUGU U UUUGAAGC
1112	UACUGUUU U CAUUUUUA	1375	AUGCUGUU U UUGAAGCA
1113	ACUGUUUU C AUUUUUA	1376	UGCUGUUU U UGAAGCAU
1116	GUUUUCAU U UUUAAGG	1377	GUGUUUUU U GAAGCAUU
1117	UUUUCAUU U UUAAGGA	1385	UGAAGCAU U UGGGUUUU
1118	UUUCAUUU U UAAAGGAA	1386	GAAGCAUU U GGGUUUUU
1119	UUCAUUUU U AAAGGAAC	1391	AUUUGGGU U UUUCUAUU
1120	UCAUUUUU A AAGGAACU	1392	UUUGGGUU U UUCUAUUU
1129	AAGGAACU C AGUUCUGG	1393	UUGGGUUU U UCUAUUUC
1133	AACUCAGU U CUGGGCCA	1394	UGGGUUUU U CUUUUUUU
1134	ACUCAGUU C UGGGCCAU	1395	GGGUUUUU C UAUUUUUU
1143	UGGGCCAU U AGAGGAAA	1397	GUUUUUUU A UUUCUCCA
1144	GGGCCAUU A GAGGAAAU	1399	UUUUUUUU U UCUUCAGU
1158	AAUGAGGU A CAAGCUGG	1400	UUUCUAUU U CUUCAGUG
1168	AAGCUGGU U ACCCAAGA	1401	UUCUAUUU C UUCAGUGG
1169	AGCUGGUU A CCCAAGAA	1403	CUUUUUUU U CAGUGGAU
1182	AGAAGCAU C CACACCCU	1404	UAUUUUUU C AGUGGAUC
1195	CCCUGGGU U UCCCUUCA	1412	CAGUGGAU C UUCACAGU
1196	CCUGGGUU U CCUUUCAA	1414	GUGGAUUC U CACAGUCG
1197	CUGGGUUU C CCUUCAAC	1415	UGGAUUCU C ACAGUCGG
1201	GUUUCCCU U CAACCAUA	1421	UUCACAGU C GGAGUUUG
1202	UUUCCCUU C AACCAUAA	1427	GUGGGAGU U UGACCCAA
1209	UCAACCAU A AGAAAAAU	1428	UCGGAGUU U GACCCAAA
1218	AGAAAAAU U GAUGCUGC	1458	ACACAUGU U UUGAAGAG
1230	GCUGCCAU U UCUGAUAA	1459	CACAUGUU U UGAAGAGC
1231	CUGCCAUU U CUGAUUAG	1460	ACAUGUUU U GAAGAGCA
1232	UGCCAUUU C UGAUAAGG	1478	CAGCUGUU U UCAGUGUU
1237	UUUCUGAU A AGGAAAGG	1479	AGCUGGUU U CAGUGUUA
1256	GAAAACAU A CUUCUUUG	1480	GUCUGGUU C AGUGUUAG
1259	AACAUAUU U CUUUGUGG	1486	UUCAGUGU U AGGAGGGG
1260	ACAUAUUU C UUUGUGGA	1487	UCAGUGUU A GGAGGGGU
1262	AUACUUUU U UGUGGAAG	1498	AGGGGUGU A UAGAAGGC
1263	UACUUUUU U GUGGAAGA	1500	GGGUGUUA A GAAGGCAC
1277	AGACAAAU A CUGGAGGU	1519	AUGAAUGU U UUAUAUGA
1286	CUGGAGGU U UGAUGAGA	1520	UGAAUGUU U UAAUAUGA
1287	UGGAGGUU U GAUGAGAA	1521	GAAUGUUU U AAAUGAAC
1304	GAGACAGU C CCUGGAGC	1522	AAUGUUUU A AAUGAACC
1319	GCCAGGCU U UCCCAGAC	1532	AUGAACCU A AUUGUUA
1320	CCAGGCUU U CCCAGACA	1535	AACCUAAU U GUUCAACA
1321	CAGGCUUU C CCAGACAU	1538	CUAAUUGU U CAACACUU
1330	CCAGACAU A UAGCAGAA	1539	UAAUUGUU C AACACUUA
1332	AGACAUUU A GCAGAAGA	1546	UCAACACU U AGGACUUU
1343	AGAAGACU U UCCAGGAA	1547	CAACACUU A GGACUUUG
1344	GAAGACUU U CCAGGAAU	1553	UUAGGACU U UGUGAGUU
1345	AAGACUUU C CAGGAUUU	1554	UAGGACUU U GUGAGUUG
1353	CCAGGAUU U AAUCCAAA	1561	UUGUGAGU U GAAGUGGC
1354	CAGGAUUU A AUCCAAAG	1571	AAGUGGCU C AUUUUUCU
1357	GAAUUAAU C CAAAGAUC	1574	UGGCUCAU U UUCUCCUG
1365	CCAAAGAU C GAUGCUGU	1575	GGCUCAUU U UCUCUCCU

1576	GCUCAUUU U CUCCUGCA
1577	CUCAUUUU C UCCUGCAU
1579	CAUUUUCU C CUGCAUAU
1586	UCCUGCAU A UGCUUGUA
1602	AUGGGAAU C UCGAGCAU
1604	GGGAUUCU C GAGCAUGA
1620	AACUGUGU A UCUAACUG
1622	CUGUGUAU C UAAACUGA
1624	GUGUAUCU A ACUGGACU
1633	ACUGGACU U UGCACAUU
1634	CUGGACUU U GCACAUUG
1641	UUGCACAU C GUUACGGG
1644	CACAUUGU U ACGGGUGU
1645	ACAUUGUU A CGGGUGUU
1653	ACGGGUGU U CAAACAGG
1654	CGGGUGUU C AAACAGGC
1670	CUGCUGCU U AGCUUGCA
1671	UGCUGCUU A GCUUGCAC
1675	GCUUAGCU U GCACUUGA
1681	CUUGCACU U GAUCACAU
1685	CACUUGAU C ACAUGGAA
1701	AGGGAGCU U CCACGAGA
1702	GGGAGCUU C CACGAGAC
1720	GGGGAAGU A CUCAUGUG
1723	GAAGUACU C AUGUGUGA
1744	CGAGUGAU U GUGUCUAU
1749	GAUUAUU C UAUGUGGA
1751	UUAUUUU A UGUGGAUU
1759	AUGUGGAU U AUUUGCCC
1760	UGUGGAUU A UUUGCCCA
1762	UGGAUUUU U UGCCCCAU
1763	GGAUUUUU U GCCCCAUA
1770	UUGCCCCA U AUUUAADA
1771	UGCCCCAU A UUUAUAUA
1773	CCCAUUUU U UAAUAAG
1774	CCAUUUUU U AAUAAGA
1775	CAUUUUUU A AUAAAGAG
1778	UAUUUUUU A AAGAGGAU
1787	AAGAGGAU U UGUCAUUU

Table AVII: Rabbit Stromelysin HH Ribozyme Sequence

nt. Position	Ribozyme Sequence
18	GCUGUCUU CUGAUGAGGCCGAAAGGCCGAA AUGCCUUG
29	CUCAGCUC CUGAUGAGGCCGAAAGGCCGAA AUGCUGUC
39	AUUGGCUU CUGAUGAGGCCGAAAGGCCGAA ACUCAGCU
61	GGUUGGAA CUGAUGAGGCCGAAAGGCCGAA AGUUUUCA
63	AGGGUUGG CUGAUGAGGCCGAAAGGCCGAA AGAGUUUU
64	CAGGGUUG CUGAUGAGGCCGAAAGGCCGAA AAGAGUUU
75	CACAGCAG CUGAUGAGGCCGAAAGGCCGAA AGCAGGGU
93	GCUGAGCA CUGAUGAGGCCGAAAGGCCGAA AGCGCCAC
94	GGCUGAGC CUGAUGAGGCCGAAAGGCCGAA AAGCGCCA
98	GAUAGGCU CUGAUGAGGCCGAAAGGCCGAA AGCAAAGC
104	CCAGUGGA CUGAUGAGGCCGAAAGGCCGAA AGGCUGAG
106	AUCCAGUG CUGAUGAGGCCGAAAGGCCGAA AUAGGCUG
122	CAUCCCUU CUGAUGAGGCCGAAAGGCCGAA AGGCUCCA
153	UGCUGAAG CUGAUGAGGCCGAAAGGCCGAA AGGUCCAU
154	UUGCUGAA CUGAUGAGGCCGAAAGGCCGAA AAGGUCCA
156	UAUUGCUG CUGAUGAGGCCGAAAGGCCGAA AGAAGGUC
157	AUAUUGCU CUGAUGAGGCCGAAAGGCCGAA AAGAAGGU
164	UUUCCAGA CUGAUGAGGCCGAAAGGCCGAA AUUGCUGA
166	GUUUUCCA CUGAUGAGGCCGAAAGGCCGAA AUAUUGCU
176	GGUUGUAG CUGAUGAGGCCGAAAGGCCGAA AGUUUUCC
179	CAAGGUUG CUGAUGAGGCCGAAAGGCCGAA AGUAGUUU
186	UCUUUUUC CUGAUGAGGCCGAAAGGCCGAA AGGUUGUA
206	UUUUAACA CUGAUGAGGCCGAAAGGCCGAA ACUGUUUC
207	CUUUUAAC CUGAUGAGGCCGAAAGGCCGAA AACUGUUU
210	UUUCUUUU CUGAUGAGGCCGAAAGGCCGAA ACAAACTG
211	CUUUCUUU CUGAUGAGGCCGAAAGGCCGAA AACAACTU
226	AACAGGAC CUGAUGAGGCCGAAAGGCCGAA ACUGUCCU
229	AACAACAG CUGAUGAGGCCGAAAGGCCGAA ACUACUGU
234	UUUUUAAC CUGAUGAGGCCGAAAGGCCGAA ACAGGACT
237	AUUUUUUU CUGAUGAGGCCGAAAGGCCGAA ACAACAGG
238	GAUUUUUU CUGAUGAGGCCGAAAGGCCGAA AACAACAG
246	AUUUCUUG CUGAUGAGGCCGAAAGGCCGAA AUUUUUUU
263	AGCCAAGG CUGAUGAGGCCGAAAGGCCGAA ACUUCUGC
264	AAGCCAAG CUGAUGAGGCCGAAAGGCCGAA AACTUCUG
267	UCCAAGCC CUGAUGAGGCCGAAAGGCCGAA AGGAACTU
272	UCACCUCC CUGAUGAGGCCGAAAGGCCGAA AGCCAAGG
296	GGGUGUUG CUGAUGAGGCCGAAAGGCCGAA AGUCCAGC
315	GGCUUGCG CUGAUGAGGCCGAAAGGCCGAA AUCACCTC
336	ACAUCAGG CUGAUGAGGCCGAAAGGCCGAA ACGCCACA
337	AACAUCAG CUGAUGAGGCCGAAAGGCCGAA AACGCCAC
345	AAGUGACC CUGAUGAGGCCGAAAGGCCGAA ACAUCAGG

349 ACUGAAGU CUGAUGAGGCCGAAAGGCCGAA ACCAACAU
353 AGGUACUG CUGAUGAGGCCGAAAGGCCGAA AGUGACCA
354 AAGGUACU CUGAUGAGGCCGAAAGGCCGAA AAGUGACC
358 AGGGAAGG CUGAUGAGGCCGAAAGGCCGAA ACTUGAAGU
362 UGCCAGGG CUGAUGAGGCCGAAAGGCCGAA AGGUACUG
363 GUGCCAGG CUGAUGAGGCCGAAAGGCCGAA AAGGUACU
391 AGUUAGGU CUGAUGAGGCCGAAAGGCCGAA AGUUUUUG
396 CUGUAAGU CUGAUGAGGCCGAAAGGCCGAA AGGUGAGU
400 AAUCCUGU CUGAUGAGGCCGAAAGGCCGAA AGUUAGGU
401 CAUCCUG CUGAUGAGGCCGAAAGGCCGAA AAGUUAGG
408 UAAUUCAC CUGAUGAGGCCGAAAGGCCGAA AUCCUGUA
415 CGGUGUGU CUGAUGAGGCCGAAAGGCCGAA AUUCACAA
416 CCGGUGUG CUGAUGAGGCCGAAAGGCCGAA AAUUCACA
427 UCUUGGCA CUGAUGAGGCCGAAAGGCCGAA AUCCGGUG
444 GCAGCAUC CUGAUGAGGCCGAAAGGCCGAA ACAGCAUC
456 GCUUUCUC CUGAUGAGGCCGAAAGGCCGAA AUGGCAGC
466 GACCUUCA CUGAUGAGGCCGAAAGGCCGAA AGCUUUCU
474 UCCUCCCA CUGAUGAGGCCGAAAGGCCGAA ACCUUCAG
490 CGUGAGUG CUGAUGAGGCCGAAAGGCCGAA AGUCACCU
495 GAGAAAGU CUGAUGAGGCCGAAAGGCCGAA AGUGGAGU
500 UCCUGGAG CUGAUGAGGCCGAAAGGCCGAA ACGUGAGU
501 UCCUGGA CUGAUGAGGCCGAAAGGCCGAA AACGUGAG
503 ACUUCUG CUGAUGAGGCCGAAAGGCCGAA AGAACGUG
512 CUCCUUCA CUGAUGAGGCCGAAAGGCCGAA ACUUCUG
531 GAGAUCAU CUGAUGAGGCCGAAAGGCCGAA AUGUCAGC
537 CCAAAGA CUGAUGAGGCCGAAAGGCCGAA AUCAUUAU
539 CUCCAAA CUC AGGCCGAAAGGCCGAA AGAUCAUU
541 GACUCCA CUC UGAGGCCGAAAGGCCGAA AGAGAUCA
542 GGACUCCA CUGAUGAGGCCGAAAGGCCGAA AAGAGAU
543 CGGACUCC CUGAUGAGGCCGAAAGGCCGAA AAAGAGAU
549 UGUUCUC CUGAUGAGGCCGAAAGGCCGAA ACUCCAAA
565 AGGAUUA CUGAUGAGGCCGAAAGGCCGAA AUCUCCAU
566 AAGGAUA CUGAUGAGGCCGAAAGGCCGAA AAUCCCA
567 AAAGGAAU CUGAUGAGGCCGAAAGGCCGAA AAAUCUCC
568 AAAAGGAA CUGAUGAGGCCGAAAGGCCGAA AAAAUCUC
570 UCAAAAGG CUGAUGAGGCCGAAAGGCCGAA AUA AAAUC
571 AUCAAAAG CUGAUGAGGCCGAAAGGCCGAA AAUAAAUI
574 UCCAUCAA CUGAUGAGGCCGAAAGGCCGAA AGGAUUA
575 GUCCAUC CUGAUGAGGCCGAAAGGCCGAA AAGGAUA
576 GGUCCAUC CUGAUGAGGCCGAAAGGCCGAA AAAGGAU
594 UGAGCCAA CUGAUGAGGCCGAAAGGCCGAA ACAUUUCC
595 AUGAGCCA CUGAUGAGGCCGAAAGGCCGAA AACAUUUC
596 CAUGAGCC CUGAUGAGGCCGAAAGGCCGAA AAACAUUU
601 AUAAGCAU CUGAUGAGGCCGAAAGGCCGAA AGCCAAA
607 AGGUGCAU CUGAUGAGGCCGAAAGGCCGAA AGCAUGAG
608 CAGGUGCA CUGAUGAGGCCGAAAGGCCGAA AAGCAUGA
627 UCUCCAUI CUGAUGAGGCCGAAAGGCCGAA AUUCUGG
628 AUCUCCAUI CUGAUGAGGCCGAAAGGCCGAA AAUUCUG
644 CAUCAUCA CUGAUGAGGCCGAAAGGCCGAA AGUGGCA

645	UCAUCAUC	CUGAUGAGGCCGAAAGGCCGAA	AAGUGGGC
673	UCCUGUUG	CUGAUGAGGCCGAAAGGCCGAA	AUCCUUUG
688	AAGGAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUUGGUUC
689	CAAGGAU	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGUU
690	ACAAGGAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUUGGU
692	CAACAAGG	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUUG
693	GCAACAAG	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAUU
696	GCAGCAAC	CUGAUGAGGCCGAAAGGCCGAA	AGGAAUAA
699	UGAGCAGC	CUGAUGAGGCCGAAAGGCCGAA	ACAAGGAA
706	AAGCUCAU	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCAA
714	GAGUGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGTUCADG
722	GACCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGUGGCCA
730	GUGAAACA	CUGAUGAGGCCGAAAGGCCGAA	ACCCAGGG
734	CCGAGUGA	CUGAUGAGGCCGAAAGGCCGAA	ACAGACCC
735	GCCGAGUG	CUGAUGAGGCCGAAAGGCCGAA	AACAGACC
736	GGCCGAGU	CUGAUGAGGCCGAAAGGCCGAA	AAACAGAC
740	GGUUGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGUGAAAC
764	AGACUGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAUCAGC
771	GCGUUGUA	CUGAUGAGGCCGAAAGGCCGAA	ACTUGGUA
773	AGGCGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGACTUGG
782	GGUCUGUG	CUGAUGAGGCCGAAAGGCCGAA	AGGCGUUG
783	AGGUCUGU	CUGAUGAGGCCGAAAGGCCGAA	AAGGCGUU
800	AAAGCGCG	CUGAUGAGGCCGAAAGGCCGAA	ACCGGGCC
801	GAAAGGCG	CUGAUGAGGCCGAAAGGCCGAA	AACCGGGC
807	UCTUGAGA	CUGAUGAGGCCGAAAGGCCGAA	AGGCGGAA
808	AUCUUGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCGGA
809	CAUCUUGA	CUGAUGAGGCCGAAAGGCCGAA	AAAGGCGG
811	AUCAUCUU	CUGAUGAGGCCGAAAGGCCGAA	AGAAAGGC
831	AGGGAUUG	CUGAUGAGGCCGAAAGGCCGAA	AUGCCAUC
836	CAUAGAGG	CUGAUGAGGCCGAAAGGCCGAA	AUUGGAUG
840	GGUCCAUA	CUGAUGAGGCCGAAAGGCCGAA	AGGGAUUG
842	CCGUCCA	CUGAUGAGGCCGAAAGGCCGAA	AGAGGGAU
860	UAUCAGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGGG
862	GUUAUCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGGCAG
868	UCCAGAGU	CUGAUGAGGCCGAAAGGCCGAA	AUCAGGAG
872	GCACUCCA	CUGAUGAGGCCGAAAGGCCGAA	AGUUAUCA
883	AGGUUCCA	CUGAUGAGGCCGAAAGGCCGAA	AGGCACUC
894	CCUGGAGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGGUUC
898	AGAUCUUG	CUGAUGAGGCCGAAAGGCCGAA	AGGGACAG
905	GGGUCCCA	CUGAUGAGGCCGAAAGGCCGAA	AUCCUGGA
918	UCACACAU	CUGAUGAGGCCGAAAGGCCGAA	ACUGGGGU
928	CAGAUUCG	CUGAUGAGGCCGAAAGGCCGAA	AUCACACA
934	GAAGGACA	CUGAUGAGGCCGAAAGGCCGAA	AUCUGGAU
938	CAUCGAAG	CUGAUGAGGCCGAAAGGCCGAA	ACAGAUUU
941	UUGCAUCG	CUGAUGAGGCCGAAAGGCCGAA	AGGACAGA
942	AUUGCAUC	CUGAUGAGGCCGAAAGGCCGAA	AAGGACAG
951	AGAGUGCU	CUGAUGAGGCCGAAAGGCCGAA	AUUGCAUC
958	UCCCCUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUGCUGA
972	AAGAACAG	CUGAUGAGGCCGAAAGGCCGAA	AUUUCUCC

973 AAAGAACA CUGAUGAGGCCGAAAGGCCGAA AAUUUCUC
 977 CUUUAAAG CUGAUGAGGCCGAAAGGCCGAA ACAGAAUU
 978 UCUUUAAA CUGAUGAGGCCGAAAGGCCGAA AACAGAAU
 980 UGUCUUUA CUGAUGAGGCCGAAAGGCCGAA AGAACAGA
 981 CUGUCUUU CUGAUGAGGCCGAAAGGCCGAA AAGAACAG
 982 CCUGUCUU CUGAUGAGGCCGAAAGGCCGAA AAAGAACA
 992 GCCAGAAA CUGAUGAGGCCGAAAGGCCGAA ACCUGUCU
 994 GCGCCAGA CUGAUGAGGCCGAAAGGCCGAA AUACCUGU
 995 UGCGCCAG CUGAUGAGGCCGAAAGGCCGAA AAUACCUU
 996 UUGCCCA CUGAUGAGGCCGAAAGGCCGAA AAAUACCU
 1007 UCCUGAGG CUGAUGAGGCCGAAAGGCCGAA ACUUGCGC
 1011 AGAAUCCU CUGAUGAGGCCGAAAGGCCGAA AGGGACUU
 1017 GGUUCGAG CUGAUGAGGCCGAAAGGCCGAA AUCCUGAG
 1018 AGGUUCGA CUGAUGAGGCCGAAAGGCCGAA AAUCCUGA
 1020 UCAGGUUC CUGAUGAGGCCGAAAGGCCGAA AGAAUCCU
 1031 UCAAAUGA CUGAUGAGGCCGAAAGGCCGAA ACUCAGGU
 1032 AUCAAUUG CUGAUGAGGCCGAAAGGCCGAA AACUCAGG
 1033 GAUCAAUU CUGAUGAGGCCGAAAGGCCGAA AAACUCAG
 1036 AGAGAUCA CUGAUGAGGCCGAAAGGCCGAA AUGAAACU
 1037 AAGAGAUC CUGAUGAGGCCGAAAGGCCGAA AAUGAAAC
 1041 AAUGAAGA CUGAUGAGGCCGAAAGGCCGAA AUCAAUUG
 1043 AGAAUGAA CUGAUGAGGCCGAAAGGCCGAA AGAUCAA
 1045 CCAGAAUG CUGAUGAGGCCGAAAGGCCGAA AGAGAUCA
 1046 GCCAGAAU CUGAUGAGGCCGAAAGGCCGAA AAGAGAUC
 1049 AUGGCCAG CUGAUGAGGCCGAAAGGCCGAA AUGAAGAG
 1050 GAUGGCCA CUGAUGAGGCCGAAAGGCCGAA AAUGAAGA
 1058 AAGGAAGA CUGAUGAGGCCGAAAGGCCGAA AUGGCCAG
 1060 UGAAGGAA CUGAUGAGGCCGAAAGGCCGAA AGAUGGCC
 1062 GCUGAAGG CUGAUGAGGCCGAAAGGCCGAA AGAGAUGG
 1063 UGCUGAAG CUGAUGAGGCCGAAAGGCCGAA AAGAGAUG
 1066 CACUGCUG CUGAUGAGGCCGAAAGGCCGAA AGGAAGAG
 1067 CCACUGCU CUGAUGAGGCCGAAAGGCCGAA AAGGAAGA
 1085 UAACUUCA CUGAUGAGGCCGAAAGGCCGAA AUGCAGCA
 1092 CUGCUAAU CUGAUGAGGCCGAAAGGCCGAA ACUUCALU
 1093 CCUGCUAA CUGAUGAGGCCGAAAGGCCGAA AACTUCAU
 1095 UCCCUGCU CUGAUGAGGCCGAAAGGCCGAA AUAACTUC
 1096 AUCCCUGC CUGAUGAGGCCGAAAGGCCGAA AAUAACUU
 1105 GAAAACAG CUGAUGAGGCCGAAAGGCCGAA AUCCCUGC
 1110 AAAAUGAA CUGAUGAGGCCGAAAGGCCGAA ACAGUAUC
 1111 AAAAAUGA CUGAUGAGGCCGAAAGGCCGAA AACAGUAU
 1112 UAAAAAUG CUGAUGAGGCCGAAAGGCCGAA AAACAGUA
 1113 UUA AAAAU CUGAUGAGGCCGAAAGGCCGAA AAAACAGU
 1116 CCUUUAAA CUGAUGAGGCCGAAAGGCCGAA AUGAAAAAC
 1117 UCCUUUAA CUGAUGAGGCCGAAAGGCCGAA AAUGAAAA
 1118 UUCCUUUA CUGAUGAGGCCGAAAGGCCGAA AAAUGAAA
 1119 GUUCCUUU CUGAUGAGGCCGAAAGGCCGAA AAAAUGAA
 1120 AGUUCUUU CUGAUGAGGCCGAAAGGCCGAA AAAAAUGA
 1129 CCAGAACU CUGAUGAGGCCGAAAGGCCGAA AGUUCUUU
 1133 UGGCCCAG CUGAUGAGGCCGAAAGGCCGAA ACUGAGUU

1134	AUGGCCCA	CUGAUGAGGCCGAAAGGCCGAA	AACUGAGU
1143	UUUCCUCU	CUGAUGAGGCCGAAAGGCCGAA	AUGGCCCA
1144	AUUUCCUC	CUGAUGAGGCCGAAAGGCCGAA	AAUGGCCC
1158	CCAGCUUG	CUGAUGAGGCCGAAAGGCCGAA	ACCUCAUU
1168	UCUUGGU	CUGAUGAGGCCGAAAGGCCGAA	ACCAGCUU
1169	UUCUUGG	CUGAUGAGGCCGAAAGGCCGAA	AACCAGCU
1182	AGGUGUG	CUGAUGAGGCCGAAAGGCCGAA	AUGCUUCU
1195	UGAAGGGA	CUGAUGAGGCCGAAAGGCCGAA	ACCCAGGG
1196	UUGAAGGG	CUGAUGAGGCCGAAAGGCCGAA	AACCCAGG
1197	GUUGAAGG	CUGAUGAGGCCGAAAGGCCGAA	AAACCCAG
1201	UAUGGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGGGAAAC
1202	UUUUGGUU	CUGAUGAGGCCGAAAGGCCGAA	AAGGGAAA
1209	AUUUUUCU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUUGA
1218	GCAGCAUC	CUGAUGAGGCCGAAAGGCCGAA	AUUUUUCU
1230	UUUUCAGA	CUGAUGAGGCCGAAAGGCCGAA	AUGGCAGC
1231	CUUUCAG	CUGAUGAGGCCGAAAGGCCGAA	AAUGGCAG
1232	CCUUAUCA	CUGAUGAGGCCGAAAGGCCGAA	AAAUGGCA
1237	CCUUUCCU	CUGAUGAGGCCGAAAGGCCGAA	AUCAGAAA
1256	CAAAGAAG	CUGAUGAGGCCGAAAGGCCGAA	AUGUUUUC
1259	CCACAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGUAUGUU
1260	UCCACAAA	CUGAUGAGGCCGAAAGGCCGAA	AAGUAUGU
1262	CUUCCACA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGUAU
1263	UCUCCAC	CUGAUGAGGCCGAAAGGCCGAA	AAGAAGUA
1277	ACCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUUUGUCU
1286	UCUCAUCA	CUGAUGAGGCCGAAAGGCCGAA	ACCUCCAG
1287	UUCUCAUC	CUGAUGAGGCCGAAAGGCCGAA	AACCUCCA
1304	GCUCCAGG	CUGAUGAGGCCGAAAGGCCGAA	ACUGUCUC
1319	GUCUGGGA	CUGAUGAGGCCGAAAGGCCGAA	AGCCUGGC
1320	UGUCUGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGCCUGG
1321	AUGUCUGG	CUGAUGAGGCCGAAAGGCCGAA	AAAGCCUG
1330	UUCUGCUA	CUGAUGAGGCCGAAAGGCCGAA	AUGUCUGG
1332	UCUUCUGC	CUGAUGAGGCCGAAAGGCCGAA	AUAUGUCU
1343	UUCCUGGA	CUGAUGAGGCCGAAAGGCCGAA	AGUCUUCU
1344	AUUCCTUG	CUGAUGAGGCCGAAAGGCCGAA	AAGUCUUC
1345	AAUUCCTU	CUGAUGAGGCCGAAAGGCCGAA	AAAGUCUU
1353	UUUGGAUU	CUGAUGAGGCCGAAAGGCCGAA	AUUCCTUG
1354	CUUUGGAU	CUGAUGAGGCCGAAAGGCCGAA	AAUUCCTU
1357	GAUCUUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUAUUUC
1365	ACAGCAUC	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUGG
1374	GCUUCAA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCAUC
1375	UGCUUCA	CUGAUGAGGCCGAAAGGCCGAA	AACAGCAU
1376	AUGCUUCA	CUGAUGAGGCCGAAAGGCCGAA	AAACAGCA
1377	AAUGCUUC	CUGAUGAGGCCGAAAGGCCGAA	AAAACAGC
1385	AAAACCCA	CUGAUGAGGCCGAAAGGCCGAA	AUGCUUCA
1386	AAAAACCC	CUGAUGAGGCCGAAAGGCCGAA	AAUGCUUC
1391	AAUAGAAA	CUGAUGAGGCCGAAAGGCCGAA	ACCCAAAU
1392	AAAUAGAA	CUGAUGAGGCCGAAAGGCCGAA	AACCCAAA
1393	GAAAUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAACCCAA
1394	AGAAAUAG	CUGAUGAGGCCGAAAGGCCGAA	AAAACCCA

1395	AAGAAUA	CUGAUGAGGCCGAAAGGCCGAA	AAAAACC
1397	UGAAGAAA	CUGAUGAGGCCGAAAGGCCGAA	AGAAAAAC
1399	ACUGAAGA	CUGAUGAGGCCGAAAGGCCGAA	AUAGAAAA
1400	CACUGAAG	CUGAUGAGGCCGAAAGGCCGAA	AAUAGAAA
1401	CCACUGAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAGAA
1403	AUCCACUG	CUGAUGAGGCCGAAAGGCCGAA	AGAAAUAG
1404	GAUCCACU	CUGAUGAGGCCGAAAGGCCGAA	AAGAAUA
1412	ACUGUGAA	CUGAUGAGGCCGAAAGGCCGAA	AUCCACUG
1414	CGACUGUG	CUGAUGAGGCCGAAAGGCCGAA	AGAUCCAC
1415	CCGACUGU	CUGAUGAGGCCGAAAGGCCGAA	AAGAUCCA
1421	CAAACUCC	CUGAUGAGGCCGAAAGGCCGAA	ACUGUGAA
1427	UUGGGUCA	CUGAUGAGGCCGAAAGGCCGAA	ACUCCGAC
1428	UUUGGGUC	CUGAUGAGGCCGAAAGGCCGAA	AACUCCGA
1458	CUUUUCAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUUGU
1459	GCUCUUA	CUGAUGAGGCCGAAAGGCCGAA	AACAUGUG
1460	UGCUCUUC	CUGAUGAGGCCGAAAGGCCGAA	AAACAUUG
1478	AACACUGA	CUGAUGAGGCCGAAAGGCCGAA	ACCAGCUG
1479	UAACACUG	CUGAUGAGGCCGAAAGGCCGAA	AACCAGCU
1480	CUAACACTU	CUGAUGAGGCCGAAAGGCCGAA	AAACCAGC
1486	CCCUCCU	CUGAUGAGGCCGAAAGGCCGAA	ACACUGAA
1487	ACCCUCC	CUGAUGAGGCCGAAAGGCCGAA	AACACUGA
1498	GCCUUCUA	CUGAUGAGGCCGAAAGGCCGAA	ACACCCCU
1500	GUGCCUUC	CUGAUGAGGCCGAAAGGCCGAA	AUACACCC
1519	UCAUUUA	CUGAUGAGGCCGAAAGGCCGAA	ACAUUCAU
1520	UUCAUUUA	CUGAUGAGGCCGAAAGGCCGAA	AACAUUCA
1521	GUUCAUUU	CUGAUGAGGCCGAAAGGCCGAA	AAACAUUC
1522	GGUUCAUU	CUGAUGAGGCCGAAAGGCCGAA	AAACAUUC
1532	UGAACAAU	CUGAUGAGGCCGAAAGGCCGAA	AGUCCAU
1535	UGUUGAAC	CUGAUGAGGCCGAAAGGCCGAA	AUUAGGUU
1538	AAGUGUUG	CUGAUGAGGCCGAAAGGCCGAA	ACAAUUG
1539	UAAGUGUU	CUGAUGAGGCCGAAAGGCCGAA	AACAAUUA
1546	AAAGUCCU	CUGAUGAGGCCGAAAGGCCGAA	AGUGUUGA
1547	CAAAGUCC	CUGAUGAGGCCGAAAGGCCGAA	AAGUGUUG
1553	AACUCACA	CUGAUGAGGCCGAAAGGCCGAA	AGUCCUAA
1554	CAACUCAC	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCUA
1561	GCCACUUC	CUGAUGAGGCCGAAAGGCCGAA	ACUCACAA
1571	GAGAAAAU	CUGAUGAGGCCGAAAGGCCGAA	AGCCACUU
1574	CAGGAGAA	CUGAUGAGGCCGAAAGGCCGAA	AUGAGCCA
1575	GCAGGAGA	CUGAUGAGGCCGAAAGGCCGAA	AAUGAGCC
1576	UGCAGGAG	CUGAUGAGGCCGAAAGGCCGAA	AAAUAGGC
1577	AUGCAGGA	CUGAUGAGGCCGAAAGGCCGAA	AAAAUGAG
1579	AUAUGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAAAAUG
1586	UCACAGCA	CUGAUGAGGCCGAAAGGCCGAA	AUGCAGGA
1602	AUGCUCGA	CUGAUGAGGCCGAAAGGCCGAA	AUUCCTAU
1604	UCAUGCUC	CUGAUGAGGCCGAAAGGCCGAA	AGAUUCCC
1620	CAGUUAGA	CUGAUGAGGCCGAAAGGCCGAA	ACACAGUU
1622	UCCAGUUA	CUGAUGAGGCCGAAAGGCCGAA	AUACACAG
1624	AGUCCAGU	CUGAUGAGGCCGAAAGGCCGAA	AGAUACAC
1633	GAUGUGCA	CUGAUGAGGCCGAAAGGCCGAA	AGUCCAGU

1634 CGAUGUGC CUGAUGAGGCCGAAAGGCCGAA AAGUCCAG
1641 CCGUAAC CUGAUGAGGCCGAAAGGCCGAA AUGUGCAA
1644 ACACCCGU CUGAUGAGGCCGAAAGGCCGAA ACGAUGUG
1645 AACACCCG CUGAUGAGGCCGAAAGGCCGAA AACGAUGU
1653 CCUGUUUG CUGAUGAGGCCGAAAGGCCGAA ACACCCGU
1654 GCCUGUUU CUGAUGAGGCCGAAAGGCCGAA AACACCCG
1670 UGCAAGCU CUGAUGAGGCCGAAAGGCCGAA AGCAGCAG
1671 GUGCAAGC CUGAUGAGGCCGAAAGGCCGAA AAGCAGCA
1675 UCAAGUGC CUGAUGAGGCCGAAAGGCCGAA AGCTAAGC
1681 AUGUGAUC CUGAUGAGGCCGAAAGGCCGAA AGUGCAAG
1685 UUCCAUGU CUGAUGAGGCCGAAAGGCCGAA AUCAAGUG
1701 UCUCGUGG CUGAUGAGGCCGAAAGGCCGAA AGTUCCTU
1702 GUCUCGUG CUGAUGAGGCCGAAAGGCCGAA AAGCUCCTC
1720 CACAUGAG CUGAUGAGGCCGAAAGGCCGAA ACTUCCTC
1723 UCACACAU CUGAUGAGGCCGAAAGGCCGAA AGUACUUC
1744 AUAACAC CUGAUGAGGCCGAAAGGCCGAA AUCACUUG
1749 UCCACAUA CUGAUGAGGCCGAAAGGCCGAA ACACAUC
1751 AAUCCACA CUGAUGAGGCCGAAAGGCCGAA AGACACAA
1759 GGGCAAAU CUGAUGAGGCCGAAAGGCCGAA AUCCACAU
1760 UGGGCAA CUGAUGAGGCCGAAAGGCCGAA AAUCCACA
1762 AAUGGGCA CUGAUGAGGCCGAAAGGCCGAA AUAAUCCA
1763 UAAUGGGC CUGAUGAGGCCGAAAGGCCGAA AAUAUCC
1770 UAUUAAU CUGAUGAGGCCGAAAGGCCGAA AUGGGCAA
1771 UUAUAAA CUGAUGAGGCCGAAAGGCCGAA AAUGGGCA
1773 CUUUAUA CUGAUGAGGCCGAAAGGCCGAA AUAAUGGG
1774 UCUUUAU CUGAUGAGGCCGAAAGGCCGAA AAUAUUGG
1775 CUCUUAU CUGAUGAGGCCGAAAGGCCGAA AAUAUUG
1776 AUCCUCU CUGAUGAGGCCGAAAGGCCGAA AUUAAUA
1787 AAUUGACA CUGAUGAGGCCGAAAGGCCGAA AUCCUCU

Table AVIII: Human Stromelysin Hairpin Ribozyme and Target Sequences

nt. Position	RZ	Substrate
66	CGCACAGC AGAA GUAGGA ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	UCCUACU GUU GCUGUGCG
82	GCUGAGCA AGAA GCCACG ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	CGUGGCA GUU UGCUCAGC
91	AUUGGAUA AGAA GAGCAA ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	UUGGUCA GCC UAUCCAUU
192	UCCUACCA AGAA GUUUC A ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	UGAACA GUU UGUUAGGA
220	UUUUUAC AGAA GGNCCA ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	UGGUCCU GUU GUUAAAAA
328	UGACCAAC AGAA GGAACU ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	AGUUCU GAU GUUGGUCA
412	UUUGGCAA AGAA GGUGUA ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	UACACCA GAU UUGCCAAA
430	GCAGAAUC AGAA GCAUCU ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	AGAUCU GUU GAUUCUGC
439	UUCUCAAC AGAA GAAUCA ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	UGAUUCU GCU GUUGAGAA
442	GCUUUCUC AGAA GCAGAA ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	UUCUGCU GUU GAGAAAGC
691	AUUUCAUG AGAA GCNACG ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	CGUUGCU GCU CAUGAAAU
775	CGAGUCAG AGAA GGUCUG ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	ACTUCAC GAC CUGACTUG
780	GGNACCGA AGAA GGUCUG ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	CAGACCU GAC UCGGUUCC
786	ACAGGCGG AGAA GAGUCA ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	UGACTUG GUU CCGCCTGU
791	UUGAGACA AGAA GNACCG ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	CGGUUCC GCC UGUCUCAA
795	CAUUTUGA AGAA GCGCGA ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	UCCGCTU GUC UCAAGAUG
822	CAUACAGG AGAA GAAUGC ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	GCATUCA GUC CUCUUAUG
844	UCAGGGGA AGAA GGGGGA ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	UCCCCU GAC UCCCTUGA
880	UCUGGAGG AGAA GTUUC ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	GGAACTU GUC CUCUCCAG
919	AGGACCAA AGAA GGAUCA ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	UGAUCCU GCU UUGUCCUU
963	CUUUAAAG AGAA GGAUUU ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	AAAUCCU GAU CUUUAAG
1360	UCUUCAAA AGAA GCAUCA ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	UGAUCCU GUU UUGAAGA
1407	CAPACTUC AGAA GUGAAG ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	CUUCACA GUU GGAGUUG
1460	AUUAAAGC AGAA GUUACU ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	AGUACA GCU GGCUUAAU
1570	ACAGCACA AGAA GGAGAA ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	UUCUCCU GCC UGUUCCGU
1667	AGCUAAGC AGAA GCCCAU ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	AUGGGCU GCU GCUUAGCU
1670	GCAAGCTA AGAA GCAGCC ACCAGAGAAACACACGUGUGUGGUACAUUACCTUGGUA	GGCUGCU GCU UAGCUUGC

Table AIX: Rabbit Hairpin Ribozyme and Target Sequences

nt. Position	Ribozyme Sequence	Substrate
77	CCACGAC AGA GUGCA ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	UGUACU GUU GUUGUGG
99	AGUGGAUA AGAA GACCA ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	UUGCUA GCC UAUCCAU
203	UUUAPCA AGAA GUUCA ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	UGAACA GUU UGUAAAA
231	UUUUUAC AGAA GGAUA ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	UAGUUC GUU GUAAAAA
339	UACCAAC AGAA GGAUG ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	CGUUCU GAU GUUGUCA
423	CUUGGAG AGAA GGUUG ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	CACACG GAU CUUCCAG
441	GCGCAUC AGAA GCAUC ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	AGAUUC GUU GAUGGUC
702	AGCUCUG AGAA GCAUA ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	UGUUCU GUU CAUGAGU
731	CCAGUGA AGAA GACCA ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	UGGUCU GUU UCAUUGG
758	CUUGGUAC AGAA GGUUC ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	AGGUCU GAU GUACCCAG
768	GCGUGUA AGAA GGUAC ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	GUUCCA GUC UACAAAC
786	CGGOCAG AGAA GUGAG ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	CUUACA GAC CUUCCUG
797	AAAGGGG AGAA GGGCA ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	UGGUCU GUU CCGCUUU
802	UUGAGAA AGAA GAAUG ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	CGUUC GCC UUUUCA
849	GAGGACG AGAA GGUCA ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	UGGACG GCC CUUCCUC
855	UCAGGGA AGAA GGGCC ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	GGGUCU GCC UCUUUGA
891	CUUGGAG AGAA GGUUC ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	GGAAUCU GUC CUUCCAG
930	AAGGACG AGAA GGAUA ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	UGAUCA GAU CUGUCUU
935	CAUGGAG AGAA GAUUG ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	CAGAUU GUC CUUGAUG
974	CUUUAAG AGAA GAUUU ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	AAAUUC GUU CUUUAAG
1107	AAAUAGA AGAA GUAUC ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	GGAUUC GUU UUCUUUU
1130	UGGCCAG AGAA GGUUC ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	GAACUA GUU CUGGCCA
1301	GUUCAGG AGAA GUUCU ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	AGAGAC GUC CUUGGAC
1371	GUUCAGA AGAA GUAUG ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	CGAUUC GUU UUGAAGC
1418	CAACUCC AGAA GUGAG ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	CUUACA GUC GGAUUUG
1471	CUAUAAC AGAA GUUCU ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	AGCAACA GUU GGUUCCAG
1663	AGCUAAG AGAA GGUUG ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	ACAGGUU GUU GCUUAGC
1666	GCAAGUA AGAA GAGGC ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	GGUUCU GUU UAGUUUC
1733	AAUACUC AGAA GUACA ACCAGAGAACACACGUGUGGUCACAUUACCUUGUA	UGUGAC GAC GAGUAU

Table BII: Human B7-1 Hammerhead Ribozyme Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
8	AAACCCU C UGUAAAG	236	UGUGUGU U UUGUAAA
12	CCUCUGU A AAGUAAC	237	GUGUGUU U UGUAAAC
17	GUAAAGU A ACAGAAG	238	UGUGUUU U GUAAACA
26	CAGAAGU U AGAAGGG	241	GUUUUGU A AACAUCA
27	AGAAGUU A GAAGGGG	247	UAAACAU C ACUGGAG
41	GAAAGU C GCCUCUC	258	GGAGGGU C UUCUACG
46	GUCGCCU C UCUGAAG	260	AGGGUCU U CUACGUG
48	CGCCUCU C UGAAGAU	261	GGGUCUU C UACGUGA
56	UGAAGAU U ACCCAA	263	GUCUUCU A CGUGAGC
57	GAAGAUU A CCCAAAG	274	GAGCAAU U GGAUUGU
75	AAGUGAU U UGUCAUU	279	AUUGGAU U GUCAUCA
76	AGUGAUU U GUCAUUG	282	GGAUUGU C AUCAGCC
79	GAUUUGU C AUUGCUU	285	UUGUCAU C AGCCUCG
82	UUGUCAU U GCUUUAU	298	UGCCUGU U UUGCACC
86	CAUUGCU U UAUAGAC	299	GCCUGUU U UGCACCU
87	AUUGCUU U AUAGACU	300	CCUGUUU U GCACCTG
88	UUGCUUU A UAGACUG	322	CCUGGU C UUAUUG
90	GCUUUAU A GACUGUA	324	CUGGUCU U ACUUGGG
97	AGACUGU A AGAAGAG	325	UGGUCUU A CUUGGGU
110	AGAACAU C UCAGAAG	328	UCUUAU C GGUUCCA
112	AACAUCU C AGAAGUG	333	CUUGGGU C CAAAUUG
124	GUGGAGU C UUAACCU	339	UCCAAAU U GUUGGCU
126	GGAGUCU U ACCUGA	342	AAAUUGU U GGCUUUC
127	GAGUCUU A CCCUGAA	347	GUUGGCU U UCACUUU
137	CUGAAAU C AAAGGAU	348	UUGGCUU U CACUUUU
145	AAAGGAU U UAAAGAA	349	UGGCUUU C ACUUUUG
146	AAGGAUU U AAAGAAA	353	UUUCACU U UUGACCC
147	AGGAUUU A AAGAAAA	354	UUCACUU U UGACCCU
163	GUGGAUU U UUUCUUC	355	UCACUUU U GACCCUA
164	UGGAUUU U UUCUUA	362	UGACCCU A AGCAUCU
165	GGAAUUU U UCUUCAG	368	UAAGCAU C UGAAGCC
166	GAAUUUU U CUUCAGC	404	GGAACAU C ACCAUCC
167	AAUUUUU C UUCAGCA	410	UCACCAU C CAAGUGU
169	UUUUUCU U CAGCAAG	418	CAAGUGU C CAUACCU
170	UUUUCUU C AGCAAGC	422	UGUCCAU A CCUCAAU
187	UGAAACU A AAUCCAC	426	CAUACCU C AAUUUCU
191	ACUAAAU C CACAACC	430	CCUCAAU U UCUUUA
200	ACAACCU U UGGAGAC	431	CUCAAUU U CUUUCAG
201	CAACCUU U GGAGACC	432	UCAAUUU C UUUCAGC
221	ACACCCU C CAUUCUC	434	AAUUUCU U UCAGCUC
226	CUCCAAU C UCUGUGU	435	AUUUCUU U CAGCUCU
228	CCAUCU C UGUGUGU	436	UUUCUU C AGCUCUU

441	UUCAGCU C UUGGUGC	782	GUGAGCU U AUCAGUC
443	CAGCUCU U GGUGCUG	783	UGACGUU A UCAGUCA
457	GGCUGGU C UUUCUCA	785	ACGUUAU C AGUCAAA
459	CUGGUCU U UCUCACU	789	UAUCAGU C AAAGCUG
460	UGGUCUU U CUCACUU	800	GCUGACU U CCCUACA
461	GGUCUUU C UCACUUC	801	CUGACUU C CCUACAC
463	UCUUUCU C ACUUCUG	805	CUUCCCU A CACCUAG
467	UCUCACU U CUGUUCA	811	UACACCU A GUUAUUC
468	CUCACUU C UGUUCAG	814	ACCUAGU A UAUCUGA
472	CUUCUGU U CAGGUGU	816	CUAGUAU A UCUGACU
473	UUCUGUU C AGGUGUU	818	AGUAUAU C UGACUUU
480	CAGGUGU U AUCCACG	824	UCUGACU U UGAAAUU
481	AGGUGUU A UCCAGCU	825	CUGACUU U GAAAUUC
483	GUGUUAU C CACGUGA	831	UUGAAAU U CCAACUU
521	ACGCUGU C CUGUGGU	832	UGAAAUU C CAACUUC
529	CUGUGGU C ACA AUGU	838	UCCAACTU U CUAAUAU
537	ACAAUGU U UCUGUUG	839	CCAACUU C UAAUAUU
538	CA AUGUU C UGUUGA	841	AACUUCU A AUUAUAG
539	AAUGUUU C UGUUGAA	844	UUCUAAU A UUAAGAAG
543	UUUCUGU U GAAGAGC	846	CUAAUAU U AGAAGGA
562	ACAAACU C GCAUCUA	847	UAUAUAU A GAAGGAU
567	CUCGCAU C UACUGGC	855	GAAGGAU A AUUUGCU
569	CGCAUCU A CUGGCAA	858	GGAUAAU U UGCUCAA
601	GCUGACU A UGAUGUC	859	GAUAUAU U GCUCAAC
608	AUGAUGU C UGGGGAC	863	AUUUGCU C AACUCU
622	CAUGAAU A UAUGGCC	869	UCAACCU C UGGAGGU
624	UGAAUAU A UUGCCCG	877	UGGAGGU U UCCAGA
635	CCCGAGU A CUGAAC	878	GGAGGUU U UCCAGAG
651	GGACCAU C UUGAUUA	879	GAGGUUU U CCAGAGC
653	ACCAUCU U UGAUAUC	880	AGGUUUU C CAGAGCC
654	CCAUCUU U GAUAUCA	889	AGAGCCU C ACCUCUC
658	CUUGAU A UCACUAA	894	CUCACCU C UCCUGGU
660	UUGAUUA C ACUAUA	896	CACUCU C CUGGUUG
664	UAUCACU A AUAACTU	902	UCCUGGU U GGAAAAU
667	CACUAAU A ACCUCUC	920	GAAGAAU U AAUUGCC
672	AUAACCU C UCCAUUG	921	AAGAAUU A AAUGCCA
674	AACUCU C CAUUGUG	930	AUGCCAU C AACACAA
678	UCUCCAU U GUGAUCC	942	CAACAGU U UCCCAAG
684	UUGUGAU C CUGGCUC	943	AACAGUU U CCCAAGA
691	CCUGGCU C UGCGCCC	944	ACAGUUU C CCAAGAU
701	CGCCCAU C UGACGAG	952	CCAAGAU C CUGAAAC
716	GGCACAU A CGAGUGU	966	CUGAGCU C UAUGCUG
726	AGUGUGU U GUUCUGA	968	GAGCUCU A UGUGUUU
729	GUGUUGU U CUGAAGU	975	AUGCUGU U AGCAGCA
730	UGUUGUU C UGAAGUA	976	UGCUGUU A GCAGCAA
737	CUGAAGU A UGAAAAA	991	ACUGGAU U UCAAUAU
751	AGACGCU U UCAAGCG	992	CUGGAUU U CAUAUUG
752	GACGCUU U CAAGCGG	993	UGGAUUU C AAUAUGA
753	ACGCUUU C AAGCGGG	997	UUUCAAU A UGACAAC

1016	CACAGCU U CAUGUGU	1315	CAUGGAU C GUGGGGA
1017	ACAGCUU C AUGUGUC	1324	UGGGGAU C AUGAGGC
1024	CAUGUGU C UCAUCAA	1334	GAGGCAU U CUUCCCU
1026	UGUGUCU C AUCAAGU	1335	AGGCAU C UUCCCUU
1029	GUCUCAU C AAGUAUG	1337	GCAUUCU U CCCUUA
1034	AUCAAGU A UGGACAU	1338	CAUUCU C CCUUAAC
1042	UGGACAU U UAAGAGU	1342	CUUCCCU U AACAAAU
1043	GGACAUU U AAGAGUG	1343	UUCCCUU A ACAAAU
1044	GACAUUU A AGAGUGA	1350	AACAAU U UAAGCTG
1054	AGUGAAU C AGACCUU	1351	ACAAAU U AAGCUGU
1061	CAGACCU U CAACUGG	1352	CAAAUU A AGCUGUU
1062	AGACCUU C AACUGGA	1359	AAGCUGU U UUAACCA
1072	CUUGAAU A CAACCAA	1360	AGCUGUU U UAACCCAC
1090	AGAGCAU U UUCUGA	1361	GCUGUUU U ACCCACU
1091	GAGCAU U UCCUGAU	1362	CUGUUUU A CCCACUA
1092	AGCAUUU U CCUGAUA	1369	ACCCACU A CCUCACC
1093	GCAUUUU C CUGAUAA	1373	ACUACCU C ACCUUCU
1099	UCCUGAU A ACCUGCU	1378	CUCACCU U CUUAAAA
1107	ACCUGCU C CCAUCCU	1379	UCACCUU C UUA AAAA
1112	CUCCCAU C CUGGGCC	1381	ACCUUCU U AAAAACC
1122	GGGCCAU U ACCUUA	1382	CCUUCU A AAAACCU
1123	GGCCAUU A CCUUAU	1390	AAAACCU C UUUCAGA
1127	AUUACCU U AAUCUCA	1392	AACCTCU U UCAGAUU
1128	UUACCUU A AUCUCAG	1393	ACCUCU U CAGAUUA
1131	CCUUAU C UCAGUAA	1394	CCUCUUU C AGAUUA
1133	UUAUCU C AGUAAU	1399	UUCAGAU U AAGCUGA
1137	UCUCAGU A AAUGGAA	1400	UCAGAUU A AGCUGAA
1146	AUGGAAU U UUUGUGA	1412	GAACAGU U ACAAGAU
1147	UGGAAU U UUGUGAU	1413	AACAGU A CAAGAUG
1148	GGAUUU U UGUGAUA	1429	CUGGCAU C CCUCUCC
1149	GAUUUU U GUGAUUA	1433	CAUCCU C UCCUUUC
1155	UUGUGAU A UGCUGCC	1435	UCCUCU C CUUUCUC
1169	CUGACCU A CUGCUUU	1438	CUCUCCU U UCUCCCC
1175	UACUGCU U UGCCCCA	1439	UCUCCU U CUCCCCC
1176	ACUGCUU U GCCCCAA	1440	CUCCUUU C UCCCCAU
1214	GAGAGAU U GAGAAGG	1442	CCUUCU C CCCAUU
1230	AAAGUGU A CGCCUCG	1448	UCCCCAU A UGCAAUU
1239	GCCUGU A UAACAGU	1455	AUGCAU U UGCUUAA
1241	CCUGUAU A ACAGUGU	1456	UGCAAUU U GCUUAU
1249	ACAGUGU C CGCAGAA	1460	AUUUGCU U AAUGUA
1275	AAAAGAU C UGAAGGU	1461	UUUGCU A AUGUAAC
1283	UGAAGGU A GCCUCCG	1466	UUAUGU A ACCUCU
1288	GUAGCCU C CGUCAUC	1471	GUAACCU C UUCUUU
1292	CCUCCGU C AUCUCU	1473	AACUCU U CUUUUGC
1295	CCGUCAU C UCUUCUG	1474	ACCUCU C UUUUGCC
1297	GUCAUCU C UUCUGGG	1476	CUCUUCU U UUGCCAU
1299	CAUCUCU U CUGGGAU	1477	UCUUCU U UGCCAUG
1300	AUCUCU C UGGGAUA	1478	CUUCUU U GCCAUGU
1307	CUGGGAU A CAUGGAU	1486	GCCAUGU U UCCAUC

1487	CCAUGUU U CCAUUCU
1488	CAUGUUU C CAUUCUG
1492	UUUCCAU U CUGCCAU
1493	UUCCAUU C UGCCAUC
1500	CUGCCAU C UUGAAUU
1502	GCCAUCU U GAAUUGU
1507	CUUGAAU U GUCUUGU
1510	GAAUUGU C UUGUCAG
1512	AUUGUCU U GUCAGCC
1515	GUCUUGU C AGCCAAU
1523	AGCCAAU U CAUUAUC
1524	GCCAAUU C AUUAUCU
1527	AAUUCAU U AUCUADU
1528	AUUCAUU A UCUAUUA
1530	UCAUUUU C UAUUAAA
1532	AUUUAUCU A UUAACA
1534	UAUCUUAU U AAACACU
1535	AUCUUAU A AACACUA
1542	AAACACU A AUUUGAG

Table BIII: Human B7-1 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequence
8	CUUUACA CUGAUGAGGCCGAAAGGCCGAA AGGGUUU
12	GUUACUU CUGAUGAGGCCGAAAGGCCGAA ACAGAGG
17	CUUCUGU CUGAUGAGGCCGAAAGGCCGAA ACUUUAC
26	CCCUUCU CUGAUGAGGCCGAAAGGCCGAA ACTUUCG
27	CCCCUUC CUGAUGAGGCCGAAAGGCCGAA AACUUCU
41	GAGAGGC CUGAUGAGGCCGAAAGGCCGAA ACAUUUC
46	CUUCAGA CUGAUGAGGCCGAAAGGCCGAA AGGCGAC
48	AUCUUCA CUGAUGAGGCCGAAAGGCCGAA AGAGGCG
56	UUUGGGU CUGAUGAGGCCGAAAGGCCGAA AUCUUCA
57	CUUUGGG CUGAUGAGGCCGAAAGGCCGAA AAUCUUC
75	AAUGACA CUGAUGAGGCCGAAAGGCCGAA AUCACUU
76	CAUGAC CUGAUGAGGCCGAAAGGCCGAA AAUCACU
79	AAGCAAU CUGAUGAGGCCGAAAGGCCGAA ACAAUUC
82	AUAAAGC CUGAUGAGGCCGAAAGGCCGAA AUGACAA
86	GUCUAUA CUGAUGAGGCCGAAAGGCCGAA AGCAAUG
87	AGUCUAU CUGAUGAGGCCGAAAGGCCGAA AAGCAAU
88	CAGUCUA CUGAUGAGGCCGAAAGGCCGAA AAAGCAA
90	UACAGUC CUGAUGAGGCCGAAAGGCCGAA AUAAAGC
97	CUCUUCU CUGAUGAGGCCGAAAGGCCGAA ACAGUCU
110	CUUCUGA CUGAUGAGGCCGAAAGGCCGAA AUGUUCU
112	CACUUCU CUGAUGAGGCCGAAAGGCCGAA AGAUGUU
124	AGGGUAA CUGAUGAGGCCGAAAGGCCGAA ACUCCAC
126	UCAGGGU CUGAUGAGGCCGAAAGGCCGAA AGACUCC
127	UUCAGGG CUGAUGAGGCCGAAAGGCCGAA AAGACUC
137	AUCCUUU CUGAUGAGGCCGAAAGGCCGAA AUUUCAG
145	UUCUUUA CUGAUGAGGCCGAAAGGCCGAA AUCCUUU
146	UUUCUUU CUGAUGAGGCCGAAAGGCCGAA AAUCCUU
147	UUUUCUU CUGAUGAGGCCGAAAGGCCGAA AAAUCCU
163	GAAGAAA CUGAUGAGGCCGAAAGGCCGAA AUUCCAC
164	UGAAGAA CUGAUGAGGCCGAAAGGCCGAA AAUUCCA
165	CUGAAGA CUGAUGAGGCCGAAAGGCCGAA AAAUUCC
166	GCUGAAG CUGAUGAGGCCGAAAGGCCGAA AAAAUUC
167	UGCUGAA CUGAUGAGGCCGAAAGGCCGAA AAAAAUC
169	CUUGCUG CUGAUGAGGCCGAAAGGCCGAA AGAAAAA
170	GCUUCU CUGAUGAGGCCGAAAGGCCGAA AAGAAAA
187	GUGGAUU CUGAUGAGGCCGAAAGGCCGAA AGUUUCA
191	GGUUGUG CUGAUGAGGCCGAAAGGCCGAA AUUUAGU
200	GUCUCCA CUGAUGAGGCCGAAAGGCCGAA AGGUUGU
201	GGUCUCC CUGAUGAGGCCGAAAGGCCGAA AAGGUUG
221	GAGAUUG CUGAUGAGGCCGAAAGGCCGAA AGGGUGU
226	ACACAGA CUGAUGAGGCCGAAAGGCCGAA AUUGGAG

228	ACACACA	CUGAUGAGGCCGAAAGGCCGAA	AGAUUGG
236	UUUACAA	CUGAUGAGGCCGAAAGGCCGAA	ACACACA
237	GUUUACA	CUGAUGAGGCCGAAAGGCCGAA	AACACAC
238	UGUUUAC	CUGAUGAGGCCGAAAGGCCGAA	AAACACA
241	UGAUGUU	CUGAUGAGGCCGAAAGGCCGAA	ACAAAAC
247	CUCCAGU	CUGAUGAGGCCGAAAGGCCGAA	AUGUUUA
258	CGUAGAA	CUGAUGAGGCCGAAAGGCCGAA	ACCCUCC
260	CACGUAG	CUGAUGAGGCCGAAAGGCCGAA	AGACCCU
261	UCACGUA	CUGAUGAGGCCGAAAGGCCGAA	AAGACCC
263	GCUCACG	CUGAUGAGGCCGAAAGGCCGAA	AGAAGAC
274	ACAAUCC	CUGAUGAGGCCGAAAGGCCGAA	AUUGCUC
279	UGAUGAC	CUGAUGAGGCCGAAAGGCCGAA	AUCCAAU
282	GGCUGAU	CUGAUGAGGCCGAAAGGCCGAA	ACAAUCC
285	CAGGGCU	CUGAUGAGGCCGAAAGGCCGAA	AUGACAA
298	GGUGCAA	CUGAUGAGGCCGAAAGGCCGAA	ACAGGCA
299	AGGUGCA	CUGAUGAGGCCGAAAGGCCGAA	AACAGGC
300	CAGGUGC	CUGAUGAGGCCGAAAGGCCGAA	AAACAGG
322	CAAGUAA	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGG
324	CCCAAGU	CUGAUGAGGCCGAAAGGCCGAA	AGACCAG
325	ACCCAAG	CUGAUGAGGCCGAAAGGCCGAA	AAGACCA
328	UGGACCC	CUGAUGAGGCCGAAAGGCCGAA	AGUAAGA
333	CAAUUUG	CUGAUGAGGCCGAAAGGCCGAA	ACCCAAG
339	AGCCAAC	CUGAUGAGGCCGAAAGGCCGAA	AUUUGGA
342	GAAAGCC	CUGAUGAGGCCGAAAGGCCGAA	ACAAUUU
347	AAAGUGA	CUGAUGAGGCCGAAAGGCCGAA	AGCCAAC
348	AAAAGUG	CUGAUGAGGCCGAAAGGCCGAA	AAGCCAA
349	CAAAAGU	CUGAUGAGGCCGAAAGGCCGAA	AAAGCCA
353	GGGUCAA	CUGAUGAGGCCGAAAGGCCGAA	AGUGAAA
354	AGGGUCA	CUGAUGAGGCCGAAAGGCCGAA	AAGUGAA
355	UAGGGUC	CUGAUGAGGCCGAAAGGCCGAA	AAAGUGA
362	AGAUGCU	CUGAUGAGGCCGAAAGGCCGAA	AGGGUCA
368	GGCUUCA	CUGAUGAGGCCGAAAGGCCGAA	AUGCUUA
404	GGAUGGU	CUGAUGAGGCCGAAAGGCCGAA	AUGUUCU
410	ACACTUG	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGA
418	AGGUUUG	CUGAUGAGGCCGAAAGGCCGAA	ACACTUG
422	AUUGAGG	CUGAUGAGGCCGAAAGGCCGAA	AUGGACA
426	AGAAAUU	CUGAUGAGGCCGAAAGGCCGAA	AGGUUUG
430	UGAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AUUGAGG
431	CUGAAAG	CUGAUGAGGCCGAAAGGCCGAA	AAUUGAG
432	GCUGAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUUGA
434	GAGCUGA	CUGAUGAGGCCGAAAGGCCGAA	AGAAAUU
435	AGAGCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGAAAU
436	AAGAGCU	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAA
441	GCACCAA	CUGAUGAGGCCGAAAGGCCGAA	AGCUGAA
443	CAGCACC	CUGAUGAGGCCGAAAGGCCGAA	AGAGCUG
457	UGAGAAA	CUGAUGAGGCCGAAAGGCCGAA	ACCAGCC
459	AGUGAGA	CUGAUGAGGCCGAAAGGCCGAA	AGACCA
460	AAGUGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGACCA
461	GAAGUGA	CUGAUGAGGCCGAAAGGCCGAA	AAAGACC

463 CAGAAGU CUGAUGAGGCCGAAAGGCCGAA AGAAAGA
467 UGAACAG CUGAUGAGGCCGAAAGGCCGAA AGUGAGA
468 CUGAACA CUGAUGAGGCCGAAAGGCCGAA AAGUGAG
472 ACACCTUG CUGAUGAGGCCGAAAGGCCGAA ACAGAAG
473 AACACCU CUGAUGAGGCCGAAAGGCCGAA AACAGAA
480 CGUGGAU CUGAUGAGGCCGAAAGGCCGAA ACACCUG
481 ACGUGGA CUGAUGAGGCCGAAAGGCCGAA AACACCU
483 UCAOGUG CUGAUGAGGCCGAAAGGCCGAA AUAACAC
521 ACCACAG CUGAUGAGGCCGAAAGGCCGAA ACAGCGU
529 ACAUUGU CUGAUGAGGCCGAAAGGCCGAA ACCACAG
537 CAACAGA CUGAUGAGGCCGAAAGGCCGAA ACAUUGU
538 UCAACAG CUGAUGAGGCCGAAAGGCCGAA AACAUUG
539 UUCAACA CUGAUGAGGCCGAAAGGCCGAA AAACAUU
543 GCTUUC CUGAUGAGGCCGAAAGGCCGAA ACAGAAA
562 UAGAUGC CUGAUGAGGCCGAAAGGCCGAA AGUUUGU
567 GCCAGUA CUGAUGAGGCCGAAAGGCCGAA AUGCGAG
569 UUGCCAG CUGAUGAGGCCGAAAGGCCGAA AGAUGCG
601 GACAUCA CUGAUGAGGCCGAAAGGCCGAA AGUCAGC
608 GUCCCCA CUGAUGAGGCCGAAAGGCCGAA ACAUCAU
622 GGCCAUU CUGAUGAGGCCGAAAGGCCGAA AUUCAUG
624 CGGCCA CUGAUGAGGCCGAAAGGCCGAA AUAUUA
635 GUUCUUG CUGAUGAGGCCGAAAGGCCGAA ACTUCGG
651 UAUCAAA CUGAUGAGGCCGAAAGGCCGAA AUGGUCC
653 GAUAUCA CUGAUGAGGCCGAAAGGCCGAA AGAUGGU
654 UGAUAUC CUGAUGAGGCCGAAAGGCCGAA AAGAUGG
658 UUAGUGA CUGAUGAGGCCGAAAGGCCGAA AUCAAAG
660 UAUAUAGU CUGAUGAGGCCGAAAGGCCGAA AUAUCAA
664 AGGUUAU CUGAUGAGGCCGAAAGGCCGAA AGUGAUA
667 GAGAGGU CUGAUGAGGCCGAAAGGCCGAA AUUAGUG
672 CAAUGGA CUGAUGAGGCCGAAAGGCCGAA AGGUUAU
674 CACAAUG CUGAUGAGGCCGAAAGGCCGAA AGAGGUU
678 GUAUCAC CUGAUGAGGCCGAAAGGCCGAA AUGGAGA
684 GAGCCAG CUGAUGAGGCCGAAAGGCCGAA AUCACAA
691 GGGCGCA CUGAUGAGGCCGAAAGGCCGAA AGCCAGG
701 CUCGUCA CUGAUGAGGCCGAAAGGCCGAA AUGGGCG
716 ACACUCG CUGAUGAGGCCGAAAGGCCGAA AUGUGCC
726 UCAGAAC CUGAUGAGGCCGAAAGGCCGAA ACACACU
729 ACUUCAG CUGAUGAGGCCGAAAGGCCGAA ACAACAC
730 UACUUA CUGAUGAGGCCGAAAGGCCGAA AACAAAC
737 UUUUUA CUGAUGAGGCCGAAAGGCCGAA ACTUCAG
751 CGCUUGA CUGAUGAGGCCGAAAGGCCGAA AGCGUCU
752 CCGCUUG CUGAUGAGGCCGAAAGGCCGAA AAGCGUC
753 CCCGCUU CUGAUGAGGCCGAAAGGCCGAA AAAGCGU
782 GACUGAU CUGAUGAGGCCGAAAGGCCGAA ACGUCAC
783 UGACUGA CUGAUGAGGCCGAAAGGCCGAA AACGUCA
785 UUUGACTU CUGAUGAGGCCGAAAGGCCGAA AUAACGU
789 CAGCUUU CUGAUGAGGCCGAAAGGCCGAA ACUGAUA
800 UGUAGGG CUGAUGAGGCCGAAAGGCCGAA AGUCAGC
801 GUGUAGG CUGAUGAGGCCGAAAGGCCGAA AAGUCAG

805 CUAGGUG CUGAUGAGGCCGAAAGGCCGAA AGGGAAG
811 GAUAUAC CUGAUGAGGCCGAAAGGCCGAA AGGUGUA
814 UCAGAUU CUGAUGAGGCCGAAAGGCCGAA ACUAGGU
816 AGUCAGA CUGAUGAGGCCGAAAGGCCGAA AUAUAG
818 AAAGUCA CUGAUGAGGCCGAAAGGCCGAA AUAUACU
824 AAUUUCA CUGAUGAGGCCGAAAGGCCGAA AGUCAGA
825 GAAUUUC CUGAUGAGGCCGAAAGGCCGAA AAGUCAG
831 AAGUUGG CUGAUGAGGCCGAAAGGCCGAA AUUUCAA
832 GAAGUUG CUGAUGAGGCCGAAAGGCCGAA AAUUUCA
838 AUAUUAU CUGAUGAGGCCGAAAGGCCGAA AGUUGGA
839 AAUAUUA CUGAUGAGGCCGAAAGGCCGAA AAGUUGG
841 CUAUAUU CUGAUGAGGCCGAAAGGCCGAA AGAAGUU
844 CUUCUAA CUGAUGAGGCCGAAAGGCCGAA AUUAGAA
846 UCCUUCU CUGAUGAGGCCGAAAGGCCGAA AUAUUAU
847 AUCCUUC CUGAUGAGGCCGAAAGGCCGAA AAUAUUA
855 AGCAAUU CUGAUGAGGCCGAAAGGCCGAA AUCCUUC
858 UUGAGCA CUGAUGAGGCCGAAAGGCCGAA AUUAUCC
859 GUUGAGC CUGAUGAGGCCGAAAGGCCGAA AAUUAUC
863 AGAGGUU CUGAUGAGGCCGAAAGGCCGAA AGCAAUU
869 ACCUCCA CUGAUGAGGCCGAAAGGCCGAA AGGUUGA
877 UCUGGAA CUGAUGAGGCCGAAAGGCCGAA ACCUCCA
878 CUCUGGA CUGAUGAGGCCGAAAGGCCGAA AACUCC
879 GCUCUGG CUGAUGAGGCCGAAAGGCCGAA AAACUUC
880 GGCUCUG CUGAUGAGGCCGAAAGGCCGAA AAAACCU
889 GAGAGGU CUGAUGAGGCCGAAAGGCCGAA AGGCUCU
894 ACCAGGA CUGAUGAGGCCGAAAGGCCGAA AGGUGAG
896 CAACCAG CUGAUGAGGCCGAAAGGCCGAA AGAGGUG
902 AUUUUCC CUGAUGAGGCCGAAAGGCCGAA ACCAGGA
920 GGCAUUU CUGAUGAGGCCGAAAGGCCGAA AUUCUUC
921 UGGCAUU CUGAUGAGGCCGAAAGGCCGAA AAUUCUU
930 UUGUGUU CUGAUGAGGCCGAAAGGCCGAA AUGGCAU
942 CUUGGGA CUGAUGAGGCCGAAAGGCCGAA ACUGUUG
943 UCUUGGG CUGAUGAGGCCGAAAGGCCGAA AACUGUU
944 AUCUUGG CUGAUGAGGCCGAAAGGCCGAA AAACUGU
952 GUUUCAG CUGAUGAGGCCGAAAGGCCGAA AUCUUGG
966 CAGCAUA CUGAUGAGGCCGAAAGGCCGAA AGCUCAG
968 AACAGCA CUGAUGAGGCCGAAAGGCCGAA AGAGCUC
975 UGCUGCU CUGAUGAGGCCGAAAGGCCGAA ACAGCAU
976 UUGCUGC CUGAUGAGGCCGAAAGGCCGAA AACAGCA
991 AUAUUGA CUGAUGAGGCCGAAAGGCCGAA AUCCAGU
992 CAUAUUG CUGAUGAGGCCGAAAGGCCGAA AAUCCAG
993 UCAUAUU CUGAUGAGGCCGAAAGGCCGAA AAUCCA
997 GUUGUCA CUGAUGAGGCCGAAAGGCCGAA AUUGAAA
1016 ACACAUG CUGAUGAGGCCGAAAGGCCGAA AGCUGUG
1017 GACACAU CUGAUGAGGCCGAAAGGCCGAA AAGCUGU
1024 UUGAUGA CUGAUGAGGCCGAAAGGCCGAA ACACAUG
1026 ACUUGAU CUGAUGAGGCCGAAAGGCCGAA AGACACA
1029 CAUACUU CUGAUGAGGCCGAAAGGCCGAA AUGAGAC
1034 AUGUCCA CUGAUGAGGCCGAAAGGCCGAA ACUUGAU

1042	ACUCUUA	CUGAUGAGGCCGAAAGGCCGAA	AUGUCCA
1043	CACUCUU	CUGAUGAGGCCGAAAGGCCGAA	AAUGUCC
1044	UCACUCU	CUGAUGAGGCCGAAAGGCCGAA	AAAUGUC
1054	AAGGUCU	CUGAUGAGGCCGAAAGGCCGAA	AUUCACU
1061	CCAGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCUG
1062	UCCAGUU	CUGAUGAGGCCGAAAGGCCGAA	AAGGUCU
1072	UUGGUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUC CAG
1090	UCAGGAA	CUGAUGAGGCCGAAAGGCCGAA	AUGCUCU
1091	AUCAGGA	CUGAUGAGGCCGAAAGGCCGAA	AAUGCUC
1092	UAUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AAAUGCU
1093	UUAUCAG	CUGAUGAGGCCGAAAGGCCGAA	AAAAUGC
1099	AGCAGGU	CUGAUGAGGCCGAAAGGCCGAA	AUCAGGA
1107	AGGAUGG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGGU
1112	GGCCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUGGGAG
1122	UUAAGGU	CUGAUGAGGCCGAAAGGCCGAA	AUGGCC
1123	AUUAAGG	CUGAUGAGGCCGAAAGGCCGAA	AAUGGCC
1127	UGAGAUU	CUGAUGAGGCCGAAAGGCCGAA	AGGUAAU
1128	CUGAGAU	CUGAUGAGGCCGAAAGGCCGAA	AAGGUAA
1131	UUAUCGA	CUGAUGAGGCCGAAAGGCCGAA	AUUAAGG
1133	AUUUACU	CUGAUGAGGCCGAAAGGCCGAA	AGAUUAA
1137	UUCCAUU	CUGAUGAGGCCGAAAGGCCGAA	ACUGAGA
1146	UCACAAA	CUGAUGAGGCCGAAAGGCCGAA	AUUC CAU
1147	AUCACAA	CUGAUGAGGCCGAAAGGCCGAA	AAUCCA
1148	UAUCACA	CUGAUGAGGCCGAAAGGCCGAA	AAAUUCC
1149	AUAUCAC	CUGAUGAGGCCGAAAGGCCGAA	AAAAUUC
1155	GGCAGCA	CUGAUGAGGCCGAAAGGCCGAA	AUCACAA
1169	AAAGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCAG
1175	UGGGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGCAGUA
1176	UUGGGGC	CUGAUGAGGCCGAAAGGCCGAA	AAGCAGU
1214	CCUUCUC	CUGAUGAGGCCGAAAGGCCGAA	AUCUCUC
1230	CAGGGCG	CUGAUGAGGCCGAAAGGCCGAA	ACACUUU
1239	ACUGUUA	CUGAUGAGGCCGAAAGGCCGAA	ACAGGGC
1241	ACACTUG	CUGAUGAGGCCGAAAGGCCGAA	AUACAGG
1249	UUCUGCG	CUGAUGAGGCCGAAAGGCCGAA	ACACUGU
1275	ACCUUCA	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUU
1283	CGGAGGC	CUGAUGAGGCCGAAAGGCCGAA	ACCUUCA
1288	GAUGACG	CUGAUGAGGCCGAAAGGCCGAA	AGGCUAC
1292	AAGAGAU	CUGAUGAGGCCGAAAGGCCGAA	ACGGAGG
1295	CAGAAGA	CUGAUGAGGCCGAAAGGCCGAA	AUGACGG
1297	CCCAGAA	CUGAUGAGGCCGAAAGGCCGAA	AGAUGAC
1299	AUCC CAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGAUG
1300	UAUCCCA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGAU
1307	AUCCAUG	CUGAUGAGGCCGAAAGGCCGAA	AUCC CAG
1315	UCCCCAC	CUGAUGAGGCCGAAAGGCCGAA	AUCCAUG
1324	GCCUCAU	CUGAUGAGGCCGAAAGGCCGAA	AUCCCCA
1334	AGGGAAG	CUGAUGAGGCCGAAAGGCCGAA	AUGCCUC
1335	AAGGGAA	CUGAUGAGGCCGAAAGGCCGAA	AAUGCCU
1337	UUAAGGG	CUGAUGAGGCCGAAAGGCCGAA	AGAAUGC
1338	GUUAAGG	CUGAUGAGGCCGAAAGGCCGAA	AAGAAUG

1342 AUUUGUU CUGAUGAGGCCGAAAGGCCGAA AGGGAAG
1343 AAUUGUU CUGAUGAGGCCGAAAGGCCGAA AAGGGAA
1350 CAGCUUA CUGAUGAGGCCGAAAGGCCGAA AUUUGUU
1351 ACAGCTU CUGAUGAGGCCGAAAGGCCGAA AAUUGUU
1352 AACAGCU CUGAUGAGGCCGAAAGGCCGAA AAAUUG
1359 UGGGUA CUGAUGAGGCCGAAAGGCCGAA ACAGCTU
1360 GUGGGUA CUGAUGAGGCCGAAAGGCCGAA AACAGCU
1361 AGUGGGU CUGAUGAGGCCGAAAGGCCGAA AAACAGC
1362 UAGUGGG CUGAUGAGGCCGAAAGGCCGAA AAAACAG
1369 GGUGAGG CUGAUGAGGCCGAAAGGCCGAA AGUGGGU
1373 AGAAGGU CUGAUGAGGCCGAAAGGCCGAA AGGUAGU
1378 UUUUAAG CUGAUGAGGCCGAAAGGCCGAA AGGUGAG
1379 UUUUUA CUGAUGAGGCCGAAAGGCCGAA AAGGUGA
1381 GGUUUUU CUGAUGAGGCCGAAAGGCCGAA AGAAGGU
1382 AGGUUUU CUGAUGAGGCCGAAAGGCCGAA AAGAAGG
1390 UCUGAAA CUGAUGAGGCCGAAAGGCCGAA AGGUUUU
1392 AAUCUGA CUGAUGAGGCCGAAAGGCCGAA AGAGGUU
1393 UAAUCUG CUGAUGAGGCCGAAAGGCCGAA AAGAGGU
1394 UUAUCU CUGAUGAGGCCGAAAGGCCGAA AAAGAGG
1399 UCAGCUU CUGAUGAGGCCGAAAGGCCGAA AUCUGAA
1400 UUCAGCU CUGAUGAGGCCGAAAGGCCGAA AAUCUGA
1412 AUCUUGU CUGAUGAGGCCGAAAGGCCGAA ACUGUUC
1413 CAUCUUG CUGAUGAGGCCGAAAGGCCGAA AACUGUU
1429 GGAGAGG CUGAUGAGGCCGAAAGGCCGAA AUGCCAG
1433 GAAAGGA CUGAUGAGGCCGAAAGGCCGAA AGGGAUG
1435 GAGAAAG CUGAUGAGGCCGAAAGGCCGAA AGAGGGA
1438 GGGGAGA CUGAUGAGGCCGAAAGGCCGAA AGGAGAG
1439 UGGGGAG CUGAUGAGGCCGAAAGGCCGAA AAGGAGA
1440 AUGGGGA CUGAUGAGGCCGAAAGGCCGAA AAAGGAG
1442 AUAUGGG CUGAUGAGGCCGAAAGGCCGAA AGAAAGG
1448 AAUUGCA CUGAUGAGGCCGAAAGGCCGAA AUGGGGA
1455 UUAAGCA CUGAUGAGGCCGAAAGGCCGAA AUUGCAU
1456 AUUAAGC CUGAUGAGGCCGAAAGGCCGAA AAUUGCA
1460 UUAACAU CUGAUGAGGCCGAAAGGCCGAA AGCAAAU
1461 GUUACAU CUGAUGAGGCCGAAAGGCCGAA AAGCAAA
1466 AAGAGGU CUGAUGAGGCCGAAAGGCCGAA ACAUUA
1471 AAAAGAA CUGAUGAGGCCGAAAGGCCGAA AGGUUAC
1473 GCAAAAG CUGAUGAGGCCGAAAGGCCGAA AGAGGUU
1474 GGCAAAA CUGAUGAGGCCGAAAGGCCGAA AAGAGGU
1476 AUGGCAA CUGAUGAGGCCGAAAGGCCGAA AGAAGAG
1477 CAUGGCA CUGAUGAGGCCGAAAGGCCGAA AAGAAGA
1478 ACAUGGC CUGAUGAGGCCGAAAGGCCGAA AAAGAAG
1486 GAAUGGA CUGAUGAGGCCGAAAGGCCGAA ACAUGGC
1487 AGAAUGG CUGAUGAGGCCGAAAGGCCGAA AACAUUG
1488 CAGAAUG CUGAUGAGGCCGAAAGGCCGAA AAACAUG
1492 AUGGCAG CUGAUGAGGCCGAAAGGCCGAA AUGGAAA
1493 GAUGGCA CUGAUGAGGCCGAAAGGCCGAA AAUGGAA
1500 AAUUCAA CUGAUGAGGCCGAAAGGCCGAA AUGGCAG
1502 ACAAUUC CUGAUGAGGCCGAAAGGCCGAA AGAUGGC

1507	ACAAGAC	CUGAUGAGGCCGAAAGGCCGAA	AUUC AAG
1510	CUGACAA	CUGAUGAGGCCGAAAGGCCGAA	ACAAUUC
1512	GGCUGAC	CUGAUGAGGCCGAAAGGCCGAA	AGACAAU
1515	AUUGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACAAGAC
1523	GAUAAUG	CUGAUGAGGCCGAAAGGCCGAA	AUUGGCU
1524	AGAUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGC
1527	AAUAGAU	CUGAUGAGGCCGAAAGGCCGAA	AUGAAUU
1528	UAAUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAUGAAU
1530	UUUAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAAUGA
1532	UGUUUAA	CUGAUGAGGCCGAAAGGCCGAA	AGAUAAU
1534	AGUGUUU	CUGAUGAGGCCGAAAGGCCGAA	AUAGAUU
1535	UAGUGUU	CUGAUGAGGCCGAAAGGCCGAA	AAUAGAU
1542	CUCAAUU	CUGAUGAGGCCGAAAGGCCGAA	AGUGUUU

Table BIV: Mouse B7-1 Hammerhead Ribozyme Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
8	GaGUuUU a UACcUcA	108	CaUcUUU a GCAuCUG
10	guUuuAU A CCUCAAU	108	CAUcUUU a gcaUCUG
10	GUuUuAU a ccuCAAU	131	aUGCCAU C caGgcUU
14	uAUacCU c aAUAGAC	142	gCUuCUU U uUCuaCA
18	CcucAAU A gaCUCUu	142	gCuUCUU u UUCUaCa
18	CCUCaaU a gaCUCUU	143	CUuCUUU u UClaCAU
18	CcUcAAU a GaCUcuU	143	CuUcUuU u uCuAcAU
23	AuaGaCU c uUACuaG	143	CUUCUUU U uCuAcaU
25	AGACuCU U aCuAGuu	143	cUUCuUU u UCUAcau
26	GACuCUU a CuAGuuu	144	UuCuUuU U cUaCAuC
29	UCUUAU a GuuUCuc	144	UuCuuuU u cUaCAUC
29	UcUuACU a gUuuCuC	144	UUUuuUU u cuaCAUC
29	UCUUAU a guUUCUc	147	uUUUuCU a cAuCUCU
29	UCuuuAU a gUUCUC	153	uAcAuCU C ugUUCU
34	CUaGUuU c UCUuuuU	165	uCUcGAU U UuUgUGA
34	CUAGUuU c UCUuuuU	165	uCUcGAU U UuuGUGA
34	cUaGUuU c uCuUuUU	165	ucucGAU U UUUGUGA
40	ucUcUuU U UCAGgUU	166	CUCgAUU U uUgUgAG
41	cUCUuUU u caGGuUg	167	uCGAUuU u UGUGaGc
41	cuCUuUU U CAGgUUG	167	ucGauUU U UGUGAgC
42	uCUuUUU C AGgUUGu	167	UCgAUUU u UgUgAGC
56	UGAAACU c AAcCuUC	168	cGAUuUu u gUgAGCC
56	UGAAAcU C aAcCUUC	168	cgAUUUU U GUGAgcc
62	uCAACCU U caaAGAC	197	GCUccAU u GgCUcUA
62	UCaAcCU U CaAAGAc	202	aUUGGCU c UagaUUc
62	UCAACCU u caaAGAc	208	UCuAgAU U ccUGGCU
63	CAACCUU c aaAGACa	216	CCUGGCU u UcCcCau
73	aGAcAcU c UGUUcCA	217	cUGGCUU U CcCcaUc
77	acUCUgU u cCAuUUC	217	cUgGCuU u CccCAUC
78	CucUGUU c CauUUCU	217	CUGGCuU u CCcCauC
83	UucCAuU U CUGUgga	218	UGGcuUU c ccCaUCA
93	GUggAcU A AuAGgAu	218	UGGCUUU C cCcaUca
93	gUgGacU a AUAGgaU	218	UGgCuUU c cCcaUCA
93	gUGgAcU a AuAGGAU	218	ugGcUUU c CCCAucA
96	GAcuAAU a GGAUcaU	224	UCcCCAU c aUGuUCu
96	gacuAAU a gGAUcaU	224	UccCCAU c aUGuucU
101	AUaGGAU c aUCuUuA	230	UCAugUU C UccAAAag
104	GGAUCAU C uuuAgCa	232	AuGUUcU C CAaAGCa
104	GGAUCAU C UUUagcA	232	AUGuUcU c caaAGCA
106	AuCAUTU U UagcAUC	232	AugUUCU c cAAAGCa
107	UcAuCuU u AGCAUCU	241	AAAGcAU c UgAAGcu
107	uCaUCUU u AgcAuCU	241	aaAGCAU C UGAAGCu

241	AAAgcAU C UGAAGcU	556	ACCuACU c uCUuAuC
249	UGAAGcU A UGGCuUG	556	AcCuAcU c ucUUAUC
264	CAAuUgU c AGuUGaU	560	AcUcUCU U aUCAuCC
287	CACCaCU c CUcaagU	561	cUCuCUU a UcAuCCU
295	CUCaAgU u UCcaUGU	561	cuCUcuU a uCAUCCU
295	cuCAaGU U UCCAUGu	561	CUCUCuU a UCauCCu
296	uCAAgUU u ccAUgUc	566	UUaUcAU C CUGGgcC
297	CAAGUuU C CAUguCc	566	uUauCAU C CUGGGCC
297	CAaGuuU c cAUGuCC	581	UGGuCcU U UcAGAcc
314	GGCUcaU u cUUCUCu	583	guCCUUU C AgaCcGG
314	GgcUcAU U CUUCuCU	583	GuCcUUU c AGAccGg
315	GcuCAUu c UuCUcuU	598	GGCACAU A CagcUGU
315	gcuCAUu C UUCuCUU	608	gcUGUGU c GUUCaaA
317	uCAUUCU U CuCUUug	611	GUGUcgU u CAaaaGA
318	CAUUCUu C uCUUugu	611	GUGUcGU U CaaAAGa
318	CAUuCuU C UCuUugu	612	UGUcGUU C aaAAGaA
320	uUCUUCU c uuUGuGC	641	aUGaAGU u aaACaCU
320	UUCuucU C UUuGUGC	649	AAAcacU U GGCUUUA
322	CuuCUCU U uGUGCUG	649	AaaCAcU U gGCUUuA
322	CUuCuCU u UgUGCUG	655	UUggcuU u AGUAAAg
323	UUcuCUU u gUGcugC	656	UGgcUUU a GUAAAgU
336	gcUGAUU c GUCuUUC	659	CuUuaGU A AAGUugu
341	uUCGuCU u UCacAAG	664	GUaAaGU U gUCcaUC
341	UUCgucU u UcacAAG	667	AaGUUGU C caUCAA
342	UcGUCUU U CaCAagU	671	UgUCcaU C AAAGCUG
343	cgucUuU C AcAAGUG	682	gCUgAcU u CuCuACC
343	cGuCuUU c AcaAGUG	682	GCUGACU U CuCUACc
352	caAGUGU C uuCAGAu	682	GCUGacU U cuCuACc
355	gUgUcUU C AGaUGUU	683	CUGACUU C uCUACcC
382	UCcaAGU c AgUGaAA	683	CUGACUU c ucuAccC
408	gCUGCcU U GCCguuA	685	gACUuCU c UaCCCCc
414	UUGcegU U aCAACUc	685	gaCUucU c UACCCcC
414	UUGCCgU u ACAAcUc	687	CUUCuCU A CcCCcAa
421	UaCAAaU c uCcUcAU	698	ccAACAU a ACUGagu
426	CUcUCCU c aUgAAGa	698	CCaacAU A ACuGaGU
439	GaUGAGU c UGAaGaC	718	AAcCCaU C UGcAgAc
452	acCGaAU C UACUGGC	718	aaCCCAU c UGCAgac
454	CGaAUcU A CUGGCAA	729	AGACacU A AaAgGAU
484	GuGCUgU c UGUcaUU	729	agAcAcU A aaAGGAU
484	GugCUGU c UguCAuU	729	agACAcU a AaAgGAU
488	ugUcUGU C AUUGCUG	737	aaAGGAU u AccUGCU
503	gGAAaCU A aaAGuGu	737	aaAGgAU U AccUGCU
503	ggAAaCU a AAagUGU	737	aaagGAU u ACCUGCU
520	CCCGAGU A uAAGAAC	745	aCCUGcU U UGcuuCc
535	cGGAcUU U aUaUGAc	745	accUGcU u UGCUuCC
536	GGAcUUU a UaUGAcA	759	cGggGgU U uCCCAAA
538	AcUuUAU a UGACaac	759	cGgGGGU u UccCAaa
553	acuACCU a cUCUcUU	759	cGGgGGU U UcCCAaA
553	AcUaCcU a cUCUcUU	760	GggGgUU u CCCAAAG

760	gGGgGUU u cCCAAag	1060	aaaUgcU u cUGUaAG
760	GGgGGUU U cCCAAAG	1060	AAAUGCU u cUgUaAG
761	GgGGUUU c CCAaAGC	1061	AAUGcUU C UGUaagc
771	aAagccU C GCuUCUC	1080	AagcugU u UCAGAAG
771	AaAGCCU C gCUUCUC	1080	AAGCUGU U UcAgaag
776	CUCgCUU C UcUUGgu	1081	AgCuGUU u CAgaAga
776	CUCgCUU C UCuUGGU	1121	acAGcCU U ACCuUcg
778	CgCuUCU C uUGGUUG	1121	AcAgCCU u .aCCuUcG
784	UCuUGGU U GGAAAU	1121	ACagCCU u ACCUUCg
803	GAGaaUU A CCugGcA	1122	CaGcCU a cCUUCgG
803	gAGAAU A ccUGgCA	1126	CUuACCU u CgGgccU
803	gagAaUU a CCUGgcA	1127	UUaCcUU c ggGcCUG
812	cUGgCAU C AAuACgA	1127	UuACcUU c GggCCUg
812	CUGGcAU c aAuaCgA	1144	GaagCAU U AgCUGAA
816	caUCAAU A cGACAAu	1144	gaAGcaU u AGCUGAA
816	cAUCAu a cgACAAU	1145	aAgCAUU a GCUgAAC
824	CgACAAU U UCCCAgG	1160	AGAcCGU c UUCCUuu
825	gACAAUU U CCCAgGA	1162	AcCGUCU u CcUUuaG
826	ACAAUUU C CCAGGAU	1163	ccGUCUU c CUUuaGU
834	CCAGGAU C CUGAAuC	1167	cUUCcUU u AGuUCUU
841	CcUGaaU C ugAAUUG	1177	uUCUUCU c UguCCAU
841	cCUGAAU c UGAauUg	1181	UCuCuGU C CAUGUGg
850	gAAuUGU A CaCCaUu	1181	ucUCUGU c CAUGUGg
869	gccAaCU a gAUuUCA	1192	gUGGGAU A CAUGGua
869	GCCAAcU a GAUuUca	1199	aCaUGGU a UUAUGUG
869	GCCAAcU a gaUuUCA	1201	AuGgUaU u aUGUGGc
873	acUaGAU u UCAaUAc	1210	ugUGGcU C aUGAGGu
873	ACUaGAU U UCAAUAc	1210	UGuGGcU C AUGAGGu
874	CUaGAUU U CAUAACG	1223	GUacAAU c UUUCUUu
875	UaGAUUU C AAUAACGA	1225	ACAAUcU U UCUuUca
885	UAcgACU c gcAACCa	1225	ACAAUCU u uCuUuca
899	ACACCaU u aAgUgUC	1226	caAuCUU u cUuUCAG
899	ACAcCaU u AaGUGUC	1227	aAucUUU c uUUCAGC
906	UaaGUGU c UcaUuAA	1227	AAucuuU C UUUCAGc
906	uAaGUGU C UCAUuAA	1227	AAUCUuU c uUUCaGC
908	aGUGUCU C AUuAAaU	1229	ucUUUCU U UCAGCaC
911	GUCUCAU u AAaUAUG	1230	cUUUCUU U CAGCaCc
916	AUuAAaU a UGGaGAu	1252	cUgAUcU u UcggaCA
916	AUuAAaU A UGGaGAU	1274	acaAGAU a gAGuUaA
943	gAGgaCU U CAcCUGG	1310	UGAgGaU u uCuUuCC
944	AGgaCUU C AccCUGG	1312	agGAUUU c UuUcCAu
1001	UGCUCUU u GggGCAG	1314	gAUUUcU u UcCAuCA
1034	CAGucGU c gUCauCG	1316	UUUcUuU c CAuCAgG
1037	UcGUCgU C AuCguUG	1320	UUUcCaU C AGgAAGC
1043	uCAUCgU U GuCAUCA	1320	UUUCcaU c aggaAGC
1046	ucgUUGU c AuCAUCA	1339	GgCAagU u UgCUGGG
1049	uUguCaU c AuCAAau	1355	cUuUgAU U GCUUGAU
1060	aaaUGcU U CUGUaag	1437	gUGguaU A aGAAAAA
1060	AAaUgCU u cUgUaAG	1437	gUggUAU a AGAAAAa

1475	gCCUAGU c UuaCUGc
1477	CUaGUCU U ACUgcaa
1487	ugCAaCU U gAUaUGU
1491	AcuUGAU a UGUCAUg
1491	aCUUgaU a UGuCAUG
1505	gUUUGgU U ggUGUcu
1530	uGCCcUU u uCUgAAg
1531	GCccUUU u CUGAagA
1532	CcCuUuU C UGAAGAg
1532	CcCuuuU C UGAaGAG
1644	CUaUGGU u gggAUGU
1652	ggGAuGU a AaAAcGG
1652	GgGAugU a aAaAcGG
1670	aUaAUaU a AaUAuUA
1674	uAuAAAAU a UuAaaUa
1676	UaAaUAU u aAaUAAA
1677	AAauAUU a AAuaAAA
1677	AaaUAUU A AAuAaaA
1694	AGagUaU u gAGcAAA

Table BV: Mouse B7-1 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequences
8	UGAGGUA CUGAUGAGGCCGAAAGGCCGAA AAAACUC
10	AUUGAGG CUGAUGAGGCCGAAAGGCCGAA AUAAAAC
10	AUUGAGG CUGAUGAGGCCGAAAGGCCGAA AUAAAAC
14	GUCUAUU CUGAUGAGGCCGAAAGGCCGAA AGGUUAUA
18	AAGAGUC CUGAUGAGGCCGAAAGGCCGAA AUUGAGG
18	AAGAGUC CUGAUGAGGCCGAAAGGCCGAA AUUGAGG
18	AAGAGUC CUGAUGAGGCCGAAAGGCCGAA AUUGAGG
23	CUAGUAA CUGAUGAGGCCGAAAGGCCGAA AGUCUAU
25	AAACUAG CUGAUGAGGCCGAAAGGCCGAA AGAGUCU
26	AAACUAG CUGAUGAGGCCGAAAGGCCGAA AAGAGUC
29	GAGAAAC CUGAUGAGGCCGAAAGGCCGAA AGUAAGA
29	GAGAAAC CUGAUGAGGCCGAAAGGCCGAA AGUAAGA
29	GAGAAAC CUGAUGAGGCCGAAAGGCCGAA AGUAAGA
29	GAGAAAC CUGAUGAGGCCGAAAGGCCGAA AGUAAGA
34	AAAAAGA CUGAUGAGGCCGAAAGGCCGAA AAACUAG
34	AAAAAGA CUGAUGAGGCCGAAAGGCCGAA AAACUAG
34	AAAAAGA CUGAUGAGGCCGAAAGGCCGAA AAACUAG
40	AACCUGA CUGAUGAGGCCGAAAGGCCGAA AAAGAGA
41	CAACCUG CUGAUGAGGCCGAAAGGCCGAA AAAAGAG
41	CAACCUG CUGAUGAGGCCGAAAGGCCGAA AAAAGAG
42	ACAACCU CUGAUGAGGCCGAAAGGCCGAA AAAAAGA
56	GAAGGUU CUGAUGAGGCCGAAAGGCCGAA AGUUUCA
56	GAAGGUU CUGAUGAGGCCGAAAGGCCGAA AGUUUCA
62	GUCUUUG CUGAUGAGGCCGAAAGGCCGAA AGGUUGA
62	GUCUUUG CUGAUGAGGCCGAAAGGCCGAA AGGUUGA
62	GUCUUUG CUGAUGAGGCCGAAAGGCCGAA AGGUUGA
63	UGUCUUU CUGAUGAGGCCGAAAGGCCGAA AAGGUUG
73	UGGAACA CUGAUGAGGCCGAAAGGCCGAA AGUGUCU
77	GAAAUUG CUGAUGAGGCCGAAAGGCCGAA ACAGAGU
78	AGAAAUG CUGAUGAGGCCGAAAGGCCGAA AACAGAG
83	UCCACAG CUGAUGAGGCCGAAAGGCCGAA AAUGGAA
93	AUCCUAU CUGAUGAGGCCGAAAGGCCGAA AGUCCAC
93	AUCCUAU CUGAUGAGGCCGAAAGGCCGAA AGUCCAC
93	AUCCUAU CUGAUGAGGCCGAAAGGCCGAA AGUCCAC
96	AUGAUCC CUGAUGAGGCCGAAAGGCCGAA AUUAGUC
96	AUGAUCC CUGAUGAGGCCGAAAGGCCGAA AUUAGUC
101	UAAAGAU CUGAUGAGGCCGAAAGGCCGAA AUCCUAU
104	UGCUIAA CUGAUGAGGCCGAAAGGCCGAA AUGAUCC
104	UGCUIAA CUGAUGAGGCCGAAAGGCCGAA AUGAUCC
106	GAUGCUA CUGAUGAGGCCGAAAGGCCGAA AGAUGAU

107	AGAUGCU	CUGAUGAGGCCGAAAGGCCGAA	AAGAUGA
107	AGAUGCU	CUGAUGAGGCCGAAAGGCCGAA	AAGAUGA
108	CAGAUGC	CUGAUGAGGCCGAAAGGCCGAA	AAAGAUG
108	CAGAUGC	CUGAUGAGGCCGAAAGGCCGAA	AAAGAUG
131	AAGCCUG	CUGAUGAGGCCGAAAGGCCGAA	AUGGCAU
142	UGUAGAA	CUGAUGAGGCCGAAAGGCCGAA	AAGAAGC
142	UGUAGAA	CUGAUGAGGCCGAAAGGCCGAA	AAGAAGC
143	AUGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAG
143	AUGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAG
143	AUGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAG
143	AUGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAG
144	GAUGUAG	CUGAUGAGGCCGAAAGGCCGAA	AAAAGAA
144	GAUGUAG	CUGAUGAGGCCGAAAGGCCGAA	AAAAGAA
144	GAUGUAG	CUGAUGAGGCCGAAAGGCCGAA	AAAAGAA
147	AGAGAUG	CUGAUGAGGCCGAAAGGCCGAA	AGAAAAA
153	AGAAACA	CUGAUGAGGCCGAAAGGCCGAA	AGAUGUA
165	UCACAAA	CUGAUGAGGCCGAAAGGCCGAA	AUCGAGA
165	UCACAAA	CUGAUGAGGCCGAAAGGCCGAA	AUCGAGA
165	UCACAAA	CUGAUGAGGCCGAAAGGCCGAA	AUCGAGA
166	UCACAAA	CUGAUGAGGCCGAAAGGCCGAA	AAUCGAG
167	GCUCACA	CUGAUGAGGCCGAAAGGCCGAA	AAAUCCA
167	GCUCACA	CUGAUGAGGCCGAAAGGCCGAA	AAAUCCA
167	GCUCACA	CUGAUGAGGCCGAAAGGCCGAA	AAAUCCA
168	GGCUCAC	CUGAUGAGGCCGAAAGGCCGAA	AAAAUCC
168	GGCUCAC	CUGAUGAGGCCGAAAGGCCGAA	AAAAUCC
197	UAGAGCC	CUGAUGAGGCCGAAAGGCCGAA	AUGGAGC
202	GAAUCUA	CUGAUGAGGCCGAAAGGCCGAA	AGCCAAU
208	AGCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AUCUAGA
216	AUGGGGA	CUGAUGAGGCCGAAAGGCCGAA	AGCCAGG
217	GAUGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGCCAG
217	GAUGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGCCAG
217	GAUGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGCCAG
218	UGAUGGG	CUGAUGAGGCCGAAAGGCCGAA	AAAGCCA
218	UGAUGGG	CUGAUGAGGCCGAAAGGCCGAA	AAAGCCA
218	UGAUGGG	CUGAUGAGGCCGAAAGGCCGAA	AAAGCCA
218	UGAUGGG	CUGAUGAGGCCGAAAGGCCGAA	AAAGCCA
224	AGAACAU	CUGAUGAGGCCGAAAGGCCGAA	AUGGGGA
224	AGAACAU	CUGAUGAGGCCGAAAGGCCGAA	AUGGGGA
230	CUUUGGA	CUGAUGAGGCCGAAAGGCCGAA	AACAUGA
232	UGC UUUG	CUGAUGAGGCCGAAAGGCCGAA	AGAACAU
232	UGC UUUG	CUGAUGAGGCCGAAAGGCCGAA	AGAACAU
232	UGC UUUG	CUGAUGAGGCCGAAAGGCCGAA	AGAACAU
241	AGCUUCA	CUGAUGAGGCCGAAAGGCCGAA	AUGCUUU
241	AGCUUCA	CUGAUGAGGCCGAAAGGCCGAA	AUGCUUU
241	AGCUUCA	CUGAUGAGGCCGAAAGGCCGAA	AUGCUUU
249	CAAGCCA	CUGAUGAGGCCGAAAGGCCGAA	AGCUUCA
264	AUCAACU	CUGAUGAGGCCGAAAGGCCGAA	ACAAUUG
287	ACUUGAG	CUGAUGAGGCCGAAAGGCCGAA	AGUGGUG
295	ACAUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACUUGAG

295 ACAUGGA CUGAUGAGGCCGAAAGGCCGAA ACTUGAG
 296 GACAUGG CUGAUGAGGCCGAAAGGCCGAA AACUUGA
 297 GGACAUG CUGAUGAGGCCGAAAGGCCGAA AAACUUG
 297 GGACAUG CUGAUGAGGCCGAAAGGCCGAA AAACUUG
 314 AGAGAAG CUGAUGAGGCCGAAAGGCCGAA AUGAGCC
 314 AGAGAAG CUGAUGAGGCCGAAAGGCCGAA AUGAGCC
 315 AAGAGAA CUGAUGAGGCCGAAAGGCCGAA AAUGAGC
 315 AAGAGAA CUGAUGAGGCCGAAAGGCCGAA AAUGAGC
 317 CAAAGAG CUGAUGAGGCCGAAAGGCCGAA AGAAUGA
 318 ACAAGA CUGAUGAGGCCGAAAGGCCGAA AAGAAUG
 318 ACAAGA CUGAUGAGGCCGAAAGGCCGAA AAGAAUG
 320 GCACAAA CUGAUGAGGCCGAAAGGCCGAA AGAAGAA
 320 GCACAAA CUGAUGAGGCCGAAAGGCCGAA AGAAGAA
 322 CAGCACA CUGAUGAGGCCGAAAGGCCGAA AGAGAAG
 322 CAGCACA CUGAUGAGGCCGAAAGGCCGAA AGAGAAG
 323 GCAGCAC CUGAUGAGGCCGAAAGGCCGAA AAGAGAA
 336 GAAAGAC CUGAUGAGGCCGAAAGGCCGAA AAUCAGC
 341 CUUGUGA CUGAUGAGGCCGAAAGGCCGAA AGACGAA
 341 CUUGUGA CUGAUGAGGCCGAAAGGCCGAA AGACGAA
 342 ACUUGUG CUGAUGAGGCCGAAAGGCCGAA AAGACGA
 343 CACUUGU CUGAUGAGGCCGAAAGGCCGAA AAAGACG
 343 CACUUGU CUGAUGAGGCCGAAAGGCCGAA AAAGACG
 352 AUCUGAA CUGAUGAGGCCGAAAGGCCGAA ACACUUG
 355 AACAUUC CUGAUGAGGCCGAAAGGCCGAA AAGACAC
 382 UUUCACU CUGAUGAGGCCGAAAGGCCGAA ACUUGGA
 408 UAACGGC CUGAUGAGGCCGAAAGGCCGAA AGGCAGC
 414 GAGUUGU CUGAUGAGGCCGAAAGGCCGAA ACGGCAA
 414 GAGUUGU CUGAUGAGGCCGAAAGGCCGAA ACGGCAA
 421 AUGAGGA CUGAUGAGGCCGAAAGGCCGAA AGUUGUA
 426 UCUCUUA CUGAUGAGGCCGAAAGGCCGAA AGGAGAG
 439 GUCUUA CUGAUGAGGCCGAAAGGCCGAA ACUCAUC
 452 GCCAGUA CUGAUGAGGCCGAAAGGCCGAA AUUCGGU
 454 UUGCCAG CUGAUGAGGCCGAAAGGCCGAA AGAUUCG
 484 AAUGACA CUGAUGAGGCCGAAAGGCCGAA ACAGCAC
 484 AAUGACA CUGAUGAGGCCGAAAGGCCGAA ACAGCAC
 488 CAGCAAU CUGAUGAGGCCGAAAGGCCGAA ACAGACA
 503 ACACUUU CUGAUGAGGCCGAAAGGCCGAA AGUUUCC
 503 ACACUUU CUGAUGAGGCCGAAAGGCCGAA AGUUUCC
 520 GUUCUUA CUGAUGAGGCCGAAAGGCCGAA ACTUCGG
 535 GUCAUUA CUGAUGAGGCCGAAAGGCCGAA AAGUCCG
 536 UGUCAUA CUGAUGAGGCCGAAAGGCCGAA AAAGUCC
 538 GUUGUCA CUGAUGAGGCCGAAAGGCCGAA AUAAAGU
 553 AAGAGAG CUGAUGAGGCCGAAAGGCCGAA AGGUAGU
 553 AAGAGAG CUGAUGAGGCCGAAAGGCCGAA AGGUAGU
 556 GAUAAGA CUGAUGAGGCCGAAAGGCCGAA AGUAGGU
 556 GAUAAGA CUGAUGAGGCCGAAAGGCCGAA AGUAGGU
 560 GGAUGAU CUGAUGAGGCCGAAAGGCCGAA AGAGAGU
 561 AGGAUGA CUGAUGAGGCCGAAAGGCCGAA AAGAGAG
 561 AGGAUGA CUGAUGAGGCCGAAAGGCCGAA AAGAGAG

561 AGGAUGA CUGAUGAGGCCGAAAGGCCGAA AAGAGAG
566 GGCCAG CUGAUGAGGCCGAAAGGCCGAA AUGAUA
566 GGCCAG CUGAUGAGGCCGAAAGGCCGAA AUGAUA
581 GGUCUGA CUGAUGAGGCCGAAAGGCCGAA AGGACCA
583 CCGGUCU CUGAUGAGGCCGAAAGGCCGAA AAAGGAC
583 CCGGUCU CUGAUGAGGCCGAAAGGCCGAA AAAGGAC
598 ACAGCUG CUGAUGAGGCCGAAAGGCCGAA AUGUGCC
608 UUUGAAC CUGAUGAGGCCGAAAGGCCGAA ACACAGC
611 UCUUUUG CUGAUGAGGCCGAAAGGCCGAA ACGACAC
611 UCUUUUG CUGAUGAGGCCGAAAGGCCGAA ACGACAC
612 UUCUUUU CUGAUGAGGCCGAAAGGCCGAA AACGACA
641 AGUGUUU CUGAUGAGGCCGAAAGGCCGAA ACUUCAU
649 UAAAGCC CUGAUGAGGCCGAAAGGCCGAA AGUGUUU
649 UAAAGCC CUGAUGAGGCCGAAAGGCCGAA AGUGUUU
655 CUUUACU CUGAUGAGGCCGAAAGGCCGAA AAGCCAA
656 ACUUUAC CUGAUGAGGCCGAAAGGCCGAA AAAGCCA
659 ACACTU CUGAUGAGGCCGAAAGGCCGAA ACTAAAG
664 GAUGGAC CUGAUGAGGCCGAAAGGCCGAA ACUUUAC
667 UUUGAUG CUGAUGAGGCCGAAAGGCCGAA ACACTU
671 CAGCUUU CUGAUGAGGCCGAAAGGCCGAA AUGGACA
682 GGUAGAG CUGAUGAGGCCGAAAGGCCGAA AGUCAGC
682 GGUAGAG CUGAUGAGGCCGAAAGGCCGAA AGUCAGC
682 GGUAGAG CUGAUGAGGCCGAAAGGCCGAA AGUCAGC
683 GGGUAGA CUGAUGAGGCCGAAAGGCCGAA AAGUCAG
683 GGGUAGA CUGAUGAGGCCGAAAGGCCGAA AAGUCAG
685 GGGGGUA CUGAUGAGGCCGAAAGGCCGAA AGAAGUC
685 GGGGGUA CUGAUGAGGCCGAAAGGCCGAA AGAAGUC
687 UUGGGGG CUGAUGAGGCCGAAAGGCCGAA AGAGAAG
698 ACUCAGU CUGAUGAGGCCGAAAGGCCGAA AUGUUGG
698 ACUCAGU CUGAUGAGGCCGAAAGGCCGAA AUGUUGG
718 GUCUGCA CUGAUGAGGCCGAAAGGCCGAA AUGGGUU
718 GUCUGCA CUGAUGAGGCCGAAAGGCCGAA AUGGGUU
729 AUCCUUU CUGAUGAGGCCGAAAGGCCGAA AGUGUCU
729 AUCCUUU CUGAUGAGGCCGAAAGGCCGAA AGUGUCU
729 AUCCUUU CUGAUGAGGCCGAAAGGCCGAA AGUGUCU
737 AGCAGGU CUGAUGAGGCCGAAAGGCCGAA AUCCUUU
737 AGCAGGU CUGAUGAGGCCGAAAGGCCGAA AUCCUUU
737 AGCAGGU CUGAUGAGGCCGAAAGGCCGAA AUCCUUU
745 GGAAGCA CUGAUGAGGCCGAAAGGCCGAA AGCAGGU
745 GGAAGCA CUGAUGAGGCCGAAAGGCCGAA AGCAGGU
759 UUUGGGA CUGAUGAGGCCGAAAGGCCGAA ACCCCCG
759 UUUGGGA CUGAUGAGGCCGAAAGGCCGAA ACCCCCG
759 UUUGGGA CUGAUGAGGCCGAAAGGCCGAA ACCCCCG
760 CUUUGGG CUGAUGAGGCCGAAAGGCCGAA AACCCCG
760 CUUUGGG CUGAUGAGGCCGAAAGGCCGAA AACCCCG
760 CUUUGGG CUGAUGAGGCCGAAAGGCCGAA AACCCCG
761 GCUUUGG CUGAUGAGGCCGAAAGGCCGAA AAACCCG
771 GAGAAGC CUGAUGAGGCCGAAAGGCCGAA AGGCUUU
771 GAGAAGC CUGAUGAGGCCGAAAGGCCGAA AGGCUUU

776	ACCAAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGCGAG
776	ACCAAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGCGAG
778	CAACCAA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGCG
784	AUUUUCC	CUGAUGAGGCCGAAAGGCCGAA	ACCAAGA
803	UGCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AAUUCUC
803	UGCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AAUUCUC
803	UGCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AAUUCUC
812	UCGUATU	CUGAUGAGGCCGAAAGGCCGAA	AUGCCAG
812	UCGUATU	CUGAUGAGGCCGAAAGGCCGAA	AUGCCAG
816	AUUGUCG	CUGAUGAGGCCGAAAGGCCGAA	AUUGAUG
816	AUUGUCG	CUGAUGAGGCCGAAAGGCCGAA	AUUGAUG
824	CCUGGGA	CUGAUGAGGCCGAAAGGCCGAA	AUUGUCG
825	UCCUGGG	CUGAUGAGGCCGAAAGGCCGAA	AAUUGUC
826	AUCCUGG	CUGAUGAGGCCGAAAGGCCGAA	AAAUUGU
834	GAUUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCCUGG
841	CAAUUCA	CUGAUGAGGCCGAAAGGCCGAA	AUUCAGG
841	CAAUUCA	CUGAUGAGGCCGAAAGGCCGAA	AUUCAGG
850	AAUGGUG	CUGAUGAGGCCGAAAGGCCGAA	ACAAUUC
869	UGAAAUUC	CUGAUGAGGCCGAAAGGCCGAA	AGUUGGC
869	UGAAAUUC	CUGAUGAGGCCGAAAGGCCGAA	AGUUGGC
869	UGAAAUUC	CUGAUGAGGCCGAAAGGCCGAA	AGUUGGC
873	GUUUUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCUAGU
873	GUUUUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCUAGU
874	CGUAUUG	CUGAUGAGGCCGAAAGGCCGAA	AAUCUAG
875	UCGUATU	CUGAUGAGGCCGAAAGGCCGAA	AAAUCUA
885	UGGUUGC	CUGAUGAGGCCGAAAGGCCGAA	AGUCGUA
899	GACACUU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGU
899	GACACUU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUGU
906	UUAAUGA	CUGAUGAGGCCGAAAGGCCGAA	ACACUUA
906	UUAAUGA	CUGAUGAGGCCGAAAGGCCGAA	ACACUUA
908	AUUUAAU	CUGAUGAGGCCGAAAGGCCGAA	AGACACU
911	CAUUAUU	CUGAUGAGGCCGAAAGGCCGAA	AUGAGAC
916	AUCUCCA	CUGAUGAGGCCGAAAGGCCGAA	AUUUAAU
916	AUCUCCA	CUGAUGAGGCCGAAAGGCCGAA	AUUUAAU
943	CCAGGUG	CUGAUGAGGCCGAAAGGCCGAA	AGUCCUC
944	CCCAGGU	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCU
1001	CUGCCCC	CUGAUGAGGCCGAAAGGCCGAA	AAGAGCA
1034	CGAUGAC	CUGAUGAGGCCGAAAGGCCGAA	ACGACUG
1037	CAACGAU	CUGAUGAGGCCGAAAGGCCGAA	ACGACGA
1043	UGAUGAC	CUGAUGAGGCCGAAAGGCCGAA	ACGAUGA
1046	UGAUGAU	CUGAUGAGGCCGAAAGGCCGAA	ACAACGA
1049	AUUUGAU	CUGAUGAGGCCGAAAGGCCGAA	AUGACAA
1060	CUUACAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAUUU
1060	CUUACAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAUUU
1060	CUUACAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAUUU
1060	CUUACAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAUUU
1061	GCUUACA	CUGAUGAGGCCGAAAGGCCGAA	AAGCAUU
1080	CUUCUGA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCTU
1080	CUUCUGA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCTU

1081 UCUUCUG CUGAUGAGGCCGAAAGGCCGAA AACAGCU
1121 CGAAGGU CUGAUGAGGCCGAAAGGCCGAA AGGCUGU
1121 CGAAGGU CUGAUGAGGCCGAAAGGCCGAA AGGCUGU
1121 CGAAGGU CUGAUGAGGCCGAAAGGCCGAA AGGCUGU
1122 CGAAGG CUGAUGAGGCCGAAAGGCCGAA AAGGCUG
1126 AGGCCCG CUGAUGAGGCCGAAAGGCCGAA AGGUAAG
1127 CAGGCC CUGAUGAGGCCGAAAGGCCGAA AAGGUA
1127 CAGGCC CUGAUGAGGCCGAAAGGCCGAA AAGGUA
1144 UUCAGCU CUGAUGAGGCCGAAAGGCCGAA AUGCUUC
1144 UUCAGCU CUGAUGAGGCCGAAAGGCCGAA AUGCUUC
1145 GUUCAGC CUGAUGAGGCCGAAAGGCCGAA AAUGCUU
1160 AAAGGAA CUGAUGAGGCCGAAAGGCCGAA ACGGUCU
1162 CUAAGG CUGAUGAGGCCGAAAGGCCGAA AGACGGU
1163 ACUAAG CUGAUGAGGCCGAAAGGCCGAA AAGACGG
1167 AAGAACU CUGAUGAGGCCGAAAGGCCGAA AAGGAAG
1177 AUGGACA CUGAUGAGGCCGAAAGGCCGAA AGAAGAA
1181 CCACAUG CUGAUGAGGCCGAAAGGCCGAA ACAGAGA
1181 CCACAUG CUGAUGAGGCCGAAAGGCCGAA ACAGAGA
1192 UACCAUG CUGAUGAGGCCGAAAGGCCGAA AUCCAC
1199 CACAUAA CUGAUGAGGCCGAAAGGCCGAA ACCAUGU
1201 GCCACAU CUGAUGAGGCCGAAAGGCCGAA AUACCAU
1210 ACCUCAU CUGAUGAGGCCGAAAGGCCGAA AGCCACA
1210 ACCUCAU CUGAUGAGGCCGAAAGGCCGAA AGCCACA
1223 AAAGAAA CUGAUGAGGCCGAAAGGCCGAA AUUGUAC
1225 UGAAAGA CUGAUGAGGCCGAAAGGCCGAA AGAUUGU
1225 UGAAAGA CUGAUGAGGCCGAAAGGCCGAA AGAUUTU
1226 CUGAAG CUGAUGAGGCCGAAAGGCCGAA AAGA
1227 GCUGAAA CUGAUGAGGCCGAAAGGCCGAA AAAGUU
1227 GCUGAAA CUGAUGAGGCCGAAAGGCCGAA AAAGAUU
1227 GCUGAAA CUGAUGAGGCCGAAAGGCCGAA AAAGAUU
1229 GUGCUGA CUGAUGAGGCCGAAAGGCCGAA AGAAAGA
1230 GGUCUG CUGAUGAGGCCGAAAGGCCGAA AAGAAAG
1252 UGUCCA CUGAUGAGGCCGAAAGGCCGAA AGAUCAG
1274 UUAACUC CUGAUGAGGCCGAAAGGCCGAA AUUUGU
1310 GGAAAGA CUGAUGAGGCCGAAAGGCCGAA AUCCUCA
1312 AUGGAAA CUGAUGAGGCCGAAAGGCCGAA AAUUCU
1314 UGAUGGA CUGAUGAGGCCGAAAGGCCGAA AGAAAU
1316 CCUGAUG CUGAUGAGGCCGAAAGGCCGAA AAAGAAA
1320 GCUUCTU CUGAUGAGGCCGAAAGGCCGAA AUGGAAA
1320 GCUUCTU CUGAUGAGGCCGAAAGGCCGAA AUGGAAA
1339 CCCAGCA CUGAUGAGGCCGAAAGGCCGAA ACUUGCC
1355 AUAAGC CUGAUGAGGCCGAAAGGCCGAA AUCAAAG
1437 UUUUUU CUGAUGAGGCCGAAAGGCCGAA AUACCAC
1437 UUUUUU CUGAUGAGGCCGAAAGGCCGAA AUACCAC
1475 GCAGUAA CUGAUGAGGCCGAAAGGCCGAA ACTUAGG
1477 UUGCAGU CUGAUGAGGCCGAAAGGCCGAA AGACUAG
1487 ACAUAUC CUGAUGAGGCCGAAAGGCCGAA AGUUGCA
1491 CAUGACA CUGAUGAGGCCGAAAGGCCGAA AUCAAGU
1491 CAUGACA CUGAUGAGGCCGAAAGGCCGAA AUCAAGU

1505	AGACACC	CUGAUGAGGCCGAAAGGCCGAA	ACCAAAC
1530	CUUCAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGGGCA
1531	UCUUCAG	CUGAUGAGGCCGAAAGGCCGAA	AAAGGGC
1532	CUCUUCA	CUGAUGAGGCCGAAAGGCCGAA	AAAAGGG
1532	CUCUUCA	CUGAUGAGGCCGAAAGGCCGAA	AAAAGGG
1644	ACAUCCC	CUGAUGAGGCCGAAAGGCCGAA	ACCAUAG
1652	CCGUUUU	CUGAUGAGGCCGAAAGGCCGAA	ACAUCCC
1652	CCGUUUU	CUGAUGAGGCCGAAAGGCCGAA	ACAUCCC
1670	UAAUAUU	CUGAUGAGGCCGAAAGGCCGAA	AUAUUUU
1674	UAUUUAA	CUGAUGAGGCCGAAAGGCCGAA	AUUUAUA
1676	UUUAUUU	CUGAUGAGGCCGAAAGGCCGAA	AUAUUUA
1677	UUUAUUU	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUU
1677	UUUAUUU	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUU
1694	UUUGCUC	CUGAUGAGGCCGAAAGGCCGAA	AUACUCU

Table BVI: Human B7-2 Hammerhead Ribozyme Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
16	GAAAGCU U UGCUUCU	271	UAGUAGU A UUUUGGC
17	AAAGCUU U GCUUCUC	273	GUAGUAU U UUGGCAG
21	CUUUGCU U CUCUGCU	274	UAGUAUU U UGGCAGG
22	UUUGCUU C UCUGCUG	275	AGUAUUU U GGCAGGA
24	UGCUUCU C UGCUUCU	294	GAAAAACU U GGUUCUG
34	CUGCUGU A ACAGGGA	298	ACUUGGU U CUGAAUG
44	AGGGACU A GCACAGA	299	CUUGGUU C UGAAUGA
70	GUGGGGU C AUUUCCA	310	AUGAGGU A UACUUAG
73	GGGUCAU U UCCAGAU	312	GAGGUAU A CUUAGGC
74	GGUCAUU U CCAGAUU	315	GUUAUACU U AGGCAAA
75	GUCAUUU C CAGAUAU	316	UAUACUU A GGCAAAAG
81	UCCAGAU A UUAGGUC	330	GAGAAAU U UGACAGU
83	CAGAUAU U AGGUCAC	331	AGAAAUU U GACAGUG
84	AGAUUUU A GGUCACA	340	ACAGUGU U CAUUCCA
88	AUUAGGU C ACAGCAG	341	CAGUGUU C AUUCCAA
113	AAUGGAU C CCCAGUG	344	UGUUCAU U CCAAGUA
125	GUGCACTU A UGGGACU	345	GUUCAUU C CAAGUAU
137	ACUGAGU A ACAUUCU	351	UCCAAGU A UAUGGGC
142	GUAACAU U CUCUUUG	353	CAAGUAA A UGGGCG
143	UAACAUU C UCUUUGU	368	CACAAGU U UUGGCG
145	ACAUUCU C UUUGUGA	369	ACAAGUU U UGAUUGG
147	AUUCUCU U UGUGAUG	370	CAAGUUU U GAUUCGG
148	UUUCUCU U GUGAUGG	374	UUUUGAU U CGGACAG
159	AUGGCCU U CCUGCUC	375	UUUGAUU C GGACAGU
160	UGGCCUU C CUGCUCU	383	GGACAGU U GGACCCU
166	UCCUGCU C UCUGGUG	397	UGAGACU U CACAAUC
168	CUGCUCU C UGGUGCU	398	GAGACUU C ACAAUUCU
179	UGCUGCU C CUCUGAA	404	UCACAAU C UUCAGAU
182	UGCUCCU C UGAAGAU	406	ACAAUCU U CAGAUCA
190	UGAAGAU U CAAGCUU	407	CAAUCUU C AGAUCAA
191	GAAGAUU C AAGCUUA	412	UUCAGAU C AAGGACA
197	UCAAGCU U AUUUCAA	426	AAGGGCU U GUUUCAA
198	CAAGCUU A UUUCAAU	429	GGCUUGU A UCAAUGU
200	AGCUUAA U UCAAUGA	431	CUUGUAA C AAUGUAA
201	GCUUAAU U CAAUGAG	437	UCAAUUU A UCAUCCA
202	CUUAAUU C AAUGAGA	439	AAUGUAA C AUCCAUC
231	UGCCAAU U UGCAAAAC	442	GUUCAAU C CAUCACA
232	GCCAAUU U GCAAAACU	446	CAUCCAU C ACAAAAA
240	GCAAAACU C UCAAAAC	469	GAUUGAU U CGCAUCC
242	AAACUCU C AAAACCA	470	AAUGAUU C GCAUCCA
265	GUGAGCU A GUAGUAA	475	UUCGCAU C CACCAGA
268	AGCUAGU A GUUUUUU	488	GAUGAAU U CUGAACU

489	AUGAAUU C UGAACUG	721	UGUCUGU U UCAUUC
498	GAACUGU C AGUGCUU	722	GUCUGUU U CAUUC
505	CAGUGCU U GCUAACU	723	UCUGUUU C AUUC
509	GCUUGCU A ACUUCAG	726	GUUUCAU U CCUGAU
513	GCUAACU U CAGUCAA	727	UUUCAUU C CCUGAUG
514	CUAACUU C AGUCAAC	736	CUGAUGU U ACGAGCA
518	CUUCAGU C AACUGA	737	UGAUGUU A CGAGCAA
529	CUGAAAU A GUACCAA	746	GAGCAAU A UGACCAU
532	AAAUAGU A CCAUUU	754	UGACCAU C UUCUGUA
538	UACCAAU U UCUAAUA	756	ACCAUCU U CUGUAUU
539	ACCAUUU U CUAAUAU	757	CCAUCUU C UGUUUUC
540	CCAAUUU C UAAUAUA	761	CUUCUGU A UUCUGGA
542	AAUUUCU A AUUAUAC	763	UCUGUAU U CUGGAAA
545	UUCUAAU A UAACAGA	764	CUGUAUU C UGGAAAC
547	CUAAUAU A ACAGAAA	787	CGCGGCU U UUAUCUU
561	AAUGUGU A CAUAAAU	788	GCGGCUU U UAUCUUC
565	UGUACAU A AAUUUGA	789	CGGCUUU U AUUCUA
569	CAUAAAU U UGACCUG	790	GGCUUUU A UCUUCAC
570	AUAAAUU U GACCUGC	792	CUUUUAU C UUCACCU
579	ACUGCUU C AUCUAUA	794	UUUAUCU U CACCUUU
582	UGCUCAU C UAUACAC	795	UUAUCUU C ACCUUUC
584	CUCAUCU A UACACGG	800	UUCACCU U UCUCUAU
586	CAUCUAU A CACGGUU	801	UCACCUU U CUCUAUA
593	ACACGGU U ACCCAGA	802	CACCUUU C UCUAUA
594	CACGGUU A CCCAGAA	804	CCUUUCU C UAUAAG
605	AGAACCU A AGAAGAU	806	UUUCUCU A UAGAGCU
619	UGAGUGU U UUGCUAA	808	UCUCUAU A GAGCUUG
620	GAGUGUU U UGCUAAG	814	UAGAGCU U GAGGACC
621	AGUGUUU U GCUAAGA	824	GGACCCU C AGCCUCC
625	UUUGCUU A AGAACCA	830	UCAGCCU C CCCAGA
638	CAAGAAU U CAACUAU	844	ACCACAU U CCUUGGA
639	AAGAAUU C AACUAUC	845	CCACAUU C CUUGGAU
644	UUCAACU A UCGAGUA	848	CAUUCU U GGAUUAC
646	CAACUAU C GAGUAUG	853	CUUGGAU U ACAGCUG
651	AUCGAGU A UGAUGGU	854	UUGGAUU A CAGCUGU
659	UGAUGGU A UUAUGCA	862	CAGCUGU A CUUCCAA
661	AUGGUUU U AUGCAGA	865	CUGUACU U CCAACAG
662	UGGUUUU A UGCAGAA	866	UGUACUU C CAACAGU
672	CAGAAAU C UCAAGAU	874	CAACAGU U AUUAUAU
674	GAAAUUCU C AAGAUAA	875	AACAGUU A UUAUAUG
680	UCAAGAU A AUGUCAC	877	CAGUUAU U AUAUGUG
685	AUAUGU C ACAGAAC	878	AGUUAUU A UAUGUGU
696	GAACUGU A CGACGUU	880	UUAUAU A UGUGUGA
703	ACGACGU U UCCAUCA	892	UGAUGGU U UUCUGUC
704	CGACGUU U CCAUCAG	893	GAUGGUU U UCUGUCU
705	GACGUUU C CAUCAGC	894	AUGGUUU U CUGUCUA
709	UUUCCAU C AGCUUGU	895	UGGUUUU C UGUCUAA
714	AUCAGCU U GUCUGUU	899	UUUCUGU C UAAUUCU
717	AGCUUGU C UGUUUA	901	UCUGUCU A AUUCUAU

904	GUCUAAU U CUAUGGA
905	UCUAAUU C UAUGGAA
907	UAAUUCU A UGGAAAU
935	GCGGCCU C GCAACUC
942	CGCAACU C UUAUAAA
944	CAACUCU U AUAAAUG
945	AACUCUU A UAAAUGU
947	CUCUUAU A AAUGUGG
1009	AAAAAAU C CAUAUAC
1013	AAUCCAU A UACCUGA
1015	UCCAUUA A CCUGAAA
1026	GAAAGAU C UGAUGAA
1045	AGCGUGU U UUUAAAA
1046	GCGUGUU U UUUAAAG
1047	CGUGUUU U UAAAAGU
1048	GUGUUUU U AAAAGUU
1049	UGUUUUU A AAAGUUC
1055	UAAAAGU U CGAAGAC
1056	AAAAGUU C GAAGACA
1065	AAGACAU C UUCAUGC
1067	GACAUCU U CAUGCGA
1068	ACAUCUU C AUGCGAC
1085	AAGUGAU A CAUGUUU
1091	UACAUGU U UUUAAUU
1092	ACAUGUU U UUAUUUA
1093	CAUGUUU U UAAUUAA
1094	AUGUUUU U AAUUAUU
1095	UGUUUUU A AUUAAAG
1098	UUUUAAU U AAAGAGU
1099	UUUAAUU A AAGAGUA

Table BVII: Human B7-2 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequences
16	AGAAGCA CUGAUGAGGCCGAAAGGCCGAA AGCTUUC
17	GAGAAGC CUGAUGAGGCCGAAAGGCCGAA AAGCTUU
21	AGCAGAG CUGAUGAGGCCGAAAGGCCGAA AGCAAAG
22	CAGCAGA CUGAUGAGGCCGAAAGGCCGAA AAGCAAA
24	AGCAGCA CUGAUGAGGCCGAAAGGCCGAA AGAAGCA
34	UCCCTUG CUGAUGAGGCCGAAAGGCCGAA ACAGCAG
44	UCUGUGC CUGAUGAGGCCGAAAGGCCGAA AGUCCCU
70	UGGAAAU CUGAUGAGGCCGAAAGGCCGAA ACCCCAC
73	AUCUGGA CUGAUGAGGCCGAAAGGCCGAA AUGACCC
74	UAUCUGG CUGAUGAGGCCGAAAGGCCGAA AAUGACC
75	AUAUCUG CUGAUGAGGCCGAAAGGCCGAA AAUGAC
81	GACCUAA CUGAUGAGGCCGAAAGGCCGAA AUCUGGA
83	GUGACCU CUGAUGAGGCCGAAAGGCCGAA AUAUCUG
84	UGUGACC CUGAUGAGGCCGAAAGGCCGAA AAUAUCU
88	CUGCUGU CUGAUGAGGCCGAAAGGCCGAA ACCUAAU
113	CACUGGG CUGAUGAGGCCGAAAGGCCGAA AUCCAUU
125	AGUCCCA CUGAUGAGGCCGAAAGGCCGAA AGUGCAC
137	AGAAUGU CUGAUGAGGCCGAAAGGCCGAA ACUCAGU
142	CAAAGAG CUGAUGAGGCCGAAAGGCCGAA AUGUURC
143	ACAAAGA CUGAUGAGGCCGAAAGGCCGAA AAUGUUA
145	UCACAAA CUGAUGAGGCCGAAAGGCCGAA AGAAUGU
147	CAUCACA CUGAUGAGGCCGAAAGGCCGAA AGAGAAU
148	CCAUCAC CUGAUGAGGCCGAAAGGCCGAA AAGAGAA
159	GAGCAGG CUGAUGAGGCCGAAAGGCCGAA AGGCCAU
160	AGAGCAG CUGAUGAGGCCGAAAGGCCGAA AAGGCCA
166	CACCAGA CUGAUGAGGCCGAAAGGCCGAA AGCAGGA
168	AGCACCA CUGAUGAGGCCGAAAGGCCGAA AGAGCAG
179	UUCAGAG CUGAUGAGGCCGAAAGGCCGAA AGCAGCA
182	AUCUUCA CUGAUGAGGCCGAAAGGCCGAA AGGAGCA
190	AAGCTUG CUGAUGAGGCCGAAAGGCCGAA AUCUUCA
191	UAAGCTU CUGAUGAGGCCGAAAGGCCGAA AAUCUUC
197	UUGAAAU CUGAUGAGGCCGAAAGGCCGAA AGCTUGA
198	AUUGAAA CUGAUGAGGCCGAAAGGCCGAA AAGCTUG
200	UCAUUGA CUGAUGAGGCCGAAAGGCCGAA AUAAGCU
201	CUCAUUG CUGAUGAGGCCGAAAGGCCGAA AAUAAGC
202	UTCUAUU CUGAUGAGGCCGAAAGGCCGAA AAUAUAG
231	GUUUGCA CUGAUGAGGCCGAAAGGCCGAA AUUGGCA
232	AGUUUGC CUGAUGAGGCCGAAAGGCCGAA AAUUGGC
240	GUUUUGA CUGAUGAGGCCGAAAGGCCGAA AGUUUGC
242	UGUUUUU CUGAUGAGGCCGAAAGGCCGAA AGAGUUU
265	AUACUAC CUGAUGAGGCCGAAAGGCCGAA AGCUCAC

268 AAAAUA CUGAUGAGGCCGAAAGGCCGAA ACUAGCU
 271 GCCAAAA CUGAUGAGGCCGAAAGGCCGAA ACTUACUA
 273 CUGCCAA CUGAUGAGGCCGAAAGGCCGAA AUACTUAC
 274 CCUGCCA CUGAUGAGGCCGAAAGGCCGAA AAUACUA
 275 UCCUGCC CUGAUGAGGCCGAAAGGCCGAA AAAUAUCU
 294 CAGAAC CUGAUGAGGCCGAAAGGCCGAA AGUUUUC
 298 CAUUCAG CUGAUGAGGCCGAAAGGCCGAA ACCAAGU
 299 UCAUUC CUGAUGAGGCCGAAAGGCCGAA AACCAAG
 310 CUAAGUA CUGAUGAGGCCGAAAGGCCGAA ACCUCAU
 312 GCCUAAG CUGAUGAGGCCGAAAGGCCGAA AUACCUC
 315 UUUGCCU CUGAUGAGGCCGAAAGGCCGAA AGUAUAC
 316 CUUUGCC CUGAUGAGGCCGAAAGGCCGAA AAGUAUA
 330 ACUGUCA CUGAUGAGGCCGAAAGGCCGAA AUUUCUC
 331 CACUGUC CUGAUGAGGCCGAAAGGCCGAA AAUUCU
 340 UGGAUUG CUGAUGAGGCCGAAAGGCCGAA ACACUGU
 341 UUGGAU CUGAUGAGGCCGAAAGGCCGAA AACACUG
 344 UACTUGG CUGAUGAGGCCGAAAGGCCGAA AUGAACA
 345 AUACUUG CUGAUGAGGCCGAAAGGCCGAA AAUGAAC
 351 GCCCAUA CUGAUGAGGCCGAAAGGCCGAA ACUUGGA
 353 CGGCCCA CUGAUGAGGCCGAAAGGCCGAA AUACUUG
 368 GAAUCAA CUGAUGAGGCCGAAAGGCCGAA ACTUGUG
 369 CGAAUCA CUGAUGAGGCCGAAAGGCCGAA AACUUGU
 370 CCGAAUC CUGAUGAGGCCGAAAGGCCGAA AAACUUG
 374 CUGUCCG CUGAUGAGGCCGAAAGGCCGAA AUCAAAA
 375 ACUGUCC CUGAUGAGGCCGAAAGGCCGAA AAUCAAA
 383 AGGGUCC CUGAUGAGGCCGAAAGGCCGAA ACUGUCC
 397 GAUUGUG CUGAUGAGGCCGAAAGGCCGAA AGUUAUA
 398 AGAUUGU CUGAUGAGGCCGAAAGGCCGAA AAUUCUC
 404 AUCUGAA CUGAUGAGGCCGAAAGGCCGAA AUUGUGA
 406 UGAUCUG CUGAUGAGGCCGAAAGGCCGAA AGAUUGU
 407 UUGAUCU CUGAUGAGGCCGAAAGGCCGAA AAGAUGU
 412 UGUCCU CUGAUGAGGCCGAAAGGCCGAA AUCUGAA
 426 UUGAUAC CUGAUGAGGCCGAAAGGCCGAA AGCCCUU
 429 ACAUUGA CUGAUGAGGCCGAAAGGCCGAA ACAAGCC
 431 AUACAUI CUGAUGAGGCCGAAAGGCCGAA AUACAAG
 437 UGGAUGA CUGAUGAGGCCGAAAGGCCGAA ACAUUGA
 439 GAUGGAU CUGAUGAGGCCGAAAGGCCGAA AUACAUI
 442 UGUGAUG CUGAUGAGGCCGAAAGGCCGAA AUGAUAC
 446 UUUUUGU CUGAUGAGGCCGAAAGGCCGAA AUGGADG
 469 GGAUGCG CUGAUGAGGCCGAAAGGCCGAA AUCAUUC
 470 UGGAUGC CUGAUGAGGCCGAAAGGCCGAA AAUCAUI
 475 UCUGGUG CUGAUGAGGCCGAAAGGCCGAA AUGCGAA
 488 AGUUCAG CUGAUGAGGCCGAAAGGCCGAA AUUCALC
 489 CAGUUCA CUGAUGAGGCCGAAAGGCCGAA AAUUCAU
 498 AAGCACTU CUGAUGAGGCCGAAAGGCCGAA ACAGUUC
 505 AGUUAGC CUGAUGAGGCCGAAAGGCCGAA AGCACTUG
 509 CUGAAGU CUGAUGAGGCCGAAAGGCCGAA AGCAAGC
 513 UUGACUG CUGAUGAGGCCGAAAGGCCGAA AGUUAAGC
 514 GUUGACU CUGAUGAGGCCGAAAGGCCGAA AAGUUAAG

518 UCAGGUU CUGAUGAGGCCGAAAGGCCGAA ACUGAAG
529 UUGGUAC CUGAUGAGGCCGAAAGGCCGAA AUUUCAG
532 AAAUUGG CUGAUGAGGCCGAAAGGCCGAA ACUAUUU
538 UAUUAGA CUGAUGAGGCCGAAAGGCCGAA AUUGGUA
539 AUUUUAG CUGAUGAGGCCGAAAGGCCGAA AAUUGGU
540 UAUUUUA CUGAUGAGGCCGAAAGGCCGAA AAAUUGG
542 GUUAUUA CUGAUGAGGCCGAAAGGCCGAA AGAAAUU
545 UCUGUUA CUGAUGAGGCCGAAAGGCCGAA AUUAGAA
547 UUUCUGU CUGAUGAGGCCGAAAGGCCGAA AUUUUAG
561 AUUUUAG CUGAUGAGGCCGAAAGGCCGAA ACACAUU
565 UCAAAUU CUGAUGAGGCCGAAAGGCCGAA AUGUACA
569 CAGGUCA CUGAUGAGGCCGAAAGGCCGAA AUUUUAG
570 GCAGGUC CUGAUGAGGCCGAAAGGCCGAA AAUUUUA
579 UAUAGAU CUGAUGAGGCCGAAAGGCCGAA AGCAGGU
582 GUGUAUA CUGAUGAGGCCGAAAGGCCGAA AUGAGCA
584 CCGUGUA CUGAUGAGGCCGAAAGGCCGAA AGAUGAG
586 AACCGUG CUGAUGAGGCCGAAAGGCCGAA AUAGAUG
593 UCUGGGU CUGAUGAGGCCGAAAGGCCGAA ACCGUGU
594 UUCUGGG CUGAUGAGGCCGAAAGGCCGAA AACCGUG
605 AUCUUCU CUGAUGAGGCCGAAAGGCCGAA AGGUUCU
619 UUAGCAA CUGAUGAGGCCGAAAGGCCGAA ACACUCA
620 CUUAGCA CUGAUGAGGCCGAAAGGCCGAA AACACUC
621 UCUUAGC CUGAUGAGGCCGAAAGGCCGAA AAACACU
625 UGGUUCU CUGAUGAGGCCGAAAGGCCGAA AGCAAAA
638 AUAGUUG CUGAUGAGGCCGAAAGGCCGAA AUUCUUG
639 GAUAGUU CUGAUGAGGCCGAAAGGCCGAA AAUUCUU
644 UACUCGA CUGAUGAGGCCGAAAGGCCGAA AGUUGAA
646 CAUACUC CUGAUGAGGCCGAAAGGCCGAA AUAGUUG
651 ACCAUCA CUGAUGAGGCCGAAAGGCCGAA ACUCGAU
659 UGCAUAA CUGAUGAGGCCGAAAGGCCGAA ACCAUCA
661 UCTUGAU CUGAUGAGGCCGAAAGGCCGAA AUACCAU
662 UUCUGCA CUGAUGAGGCCGAAAGGCCGAA AAUACCA
672 AUCUUGA CUGAUGAGGCCGAAAGGCCGAA AUUUCUG
674 UUAUCUU CUGAUGAGGCCGAAAGGCCGAA AGAUTUC
680 GUGACAU CUGAUGAGGCCGAAAGGCCGAA AUCUUGA
685 GUUCUGU CUGAUGAGGCCGAAAGGCCGAA ACAUUUA
696 AACGUCG CUGAUGAGGCCGAAAGGCCGAA ACAGUUC
703 UGAUGGA CUGAUGAGGCCGAAAGGCCGAA ACGUCGU
704 CUGAUGG CUGAUGAGGCCGAAAGGCCGAA AACGUCG
705 GCUAUG CUGAUGAGGCCGAAAGGCCGAA AAACGUC
709 ACAAGCU CUGAUGAGGCCGAAAGGCCGAA AUGGAAA
714 AACAGAC CUGAUGAGGCCGAAAGGCCGAA AGCUGAU
717 UGAAACA CUGAUGAGGCCGAAAGGCCGAA ACAAGCU
721 GGAAUGA CUGAUGAGGCCGAAAGGCCGAA ACAGACA
722 GGGAAUG CUGAUGAGGCCGAAAGGCCGAA AACAGAC
723 AGGGAAU CUGAUGAGGCCGAAAGGCCGAA AAACAGA
726 AUCAGGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAC
727 CAUCAGG CUGAUGAGGCCGAAAGGCCGAA AAUGAAA
736 UGUCUGU CUGAUGAGGCCGAAAGGCCGAA ACAUCAG

737 UUGCUCG CUGAUGAGGCCGAAAGGCCGAA AACAUCA
746 AUGGUCA CUGAUGAGGCCGAAAGGCCGAA AUUGCUC
754 UACAGAA CUGAUGAGGCCGAAAGGCCGAA AUGGUCA
756 AAUACAG CUGAUGAGGCCGAAAGGCCGAA AGAUGGU
757 GAAUACA CUGAUGAGGCCGAAAGGCCGAA AAGAUGG
761 UCCAGAA CUGAUGAGGCCGAAAGGCCGAA ACAGAAG
763 UUUCCAG CUGAUGAGGCCGAAAGGCCGAA AUACAGA
764 GUUUGCA CUGAUGAGGCCGAAAGGCCGAA AAUACAG
787 AAGAUAA CUGAUGAGGCCGAAAGGCCGAA AGCCCGC
788 GAAGAUU CUGAUGAGGCCGAAAGGCCGAA AAGCCGC
789 UGAAGAU CUGAUGAGGCCGAAAGGCCGAA AAAGCCG
790 GUGAAGA CUGAUGAGGCCGAAAGGCCGAA AAAAGCC
792 AGGUGAA CUGAUGAGGCCGAAAGGCCGAA AUAAAAG
794 AAAGGUG CUGAUGAGGCCGAAAGGCCGAA AGAUAAA
795 GAAAGGU CUGAUGAGGCCGAAAGGCCGAA AAGAUAA
800 AUAGAGA CUGAUGAGGCCGAAAGGCCGAA AGGUGAA
801 UAUAGAG CUGAUGAGGCCGAAAGGCCGAA AAGGUGA
802 CUUAAGA CUGAUGAGGCCGAAAGGCCGAA AAAGGUG
804 CUCUAUA CUGAUGAGGCCGAAAGGCCGAA AGAAAGG
806 AGCUCUA CUGAUGAGGCCGAAAGGCCGAA AGAGAAA
808 CAAGCUC CUGAUGAGGCCGAAAGGCCGAA AUAGAGA
814 GGUCCUC CUGAUGAGGCCGAAAGGCCGAA AGCUCUA
824 GGAGGCU CUGAUGAGGCCGAAAGGCCGAA AGGGUCC
830 UCUGGGG CUGAUGAGGCCGAAAGGCCGAA AGGCUGA
844 UCCAAGG CUGAUGAGGCCGAAAGGCCGAA AUGUGGU
845 AUCCAAG CUGAUGAGGCCGAAAGGCCGAA AAUGUGG
848 GUAUCC CUGAUGAGGCCGAAAGGCCGAA AGGAAUG
853 CAGCUGU CUGAUGAGGCCGAAAGGCCGAA AUCCAAG
854 ACAGCUG CUGAUGAGGCCGAAAGGCCGAA AAUCCAA
862 UUGGAAG CUGAUGAGGCCGAAAGGCCGAA ACAGCUG
865 CUGUUGG CUGAUGAGGCCGAAAGGCCGAA AGUACAG
866 ACUGUUG CUGAUGAGGCCGAAAGGCCGAA AAGUACA
874 AUUAUAA CUGAUGAGGCCGAAAGGCCGAA ACUGUUG
875 CAUAUAA CUGAUGAGGCCGAAAGGCCGAA AACUGUU
877 CACAUUU CUGAUGAGGCCGAAAGGCCGAA AUAAUCU
878 ACACAUU CUGAUGAGGCCGAAAGGCCGAA AAUAACU
880 UCACACA CUGAUGAGGCCGAAAGGCCGAA AUAAUAA
892 GACAGAA CUGAUGAGGCCGAAAGGCCGAA ACCAUCA
893 AGACAGA CUGAUGAGGCCGAAAGGCCGAA AACCAUC
894 UAGACAG CUGAUGAGGCCGAAAGGCCGAA AAAACCU
895 UUAGACA CUGAUGAGGCCGAAAGGCCGAA AAAACCA
899 AGAAUUA CUGAUGAGGCCGAAAGGCCGAA ACAGAAA
901 AUAGAAU CUGAUGAGGCCGAAAGGCCGAA AGACAGA
904 UCCAUAU CUGAUGAGGCCGAAAGGCCGAA AUUAGAC
905 UUCCAUA CUGAUGAGGCCGAAAGGCCGAA AAUUAUA
907 AUUUGCA CUGAUGAGGCCGAAAGGCCGAA AGAAUUA
935 GAGUUGC CUGAUGAGGCCGAAAGGCCGAA AGGCCGC
942 UUUUAAA CUGAUGAGGCCGAAAGGCCGAA AGUUGCG
944 CAUUUAU CUGAUGAGGCCGAAAGGCCGAA AGAGUUG

945	ACAUUUA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGUU
947	CCACAUU	CUGAUGAGGCCGAAAGGCCGAA	AUAAGAG
1009	GUUAUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUUUUU
1013	UCAGGUA	CUGAUGAGGCCGAAAGGCCGAA	AUGGAUU
1015	UUUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AUAUGGA
1026	UUCAUCA	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUC
1045	UUUUAAA	CUGAUGAGGCCGAAAGGCCGAA	ACACGCU
1046	CUUUUAA	CUGAUGAGGCCGAAAGGCCGAA	AACACGC
1047	ACUUUUA	CUGAUGAGGCCGAAAGGCCGAA	AAACACG
1048	AACUUUU	CUGAUGAGGCCGAAAGGCCGAA	AAAACAC
1049	GAACUUU	CUGAUGAGGCCGAAAGGCCGAA	AAAAACA
1055	GUCUUUG	CUGAUGAGGCCGAAAGGCCGAA	ACUUUUA
1056	UGUCUUC	CUGAUGAGGCCGAAAGGCCGAA	AACUUUU
1065	GCAUGAA	CUGAUGAGGCCGAAAGGCCGAA	AUGUCUU
1067	UCGCAUG	CUGAUGAGGCCGAAAGGCCGAA	AGAUGUC
1068	GUCCGAU	CUGAUGAGGCCGAAAGGCCGAA	AAGAUGU
1085	AAACAUG	CUGAUGAGGCCGAAAGGCCGAA	AUCACUU
1091	AAUUAAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUGUA
1092	UAAUUAA	CUGAUGAGGCCGAAAGGCCGAA	AACAUGU
1093	UUAAUUA	CUGAUGAGGCCGAAAGGCCGAA	AAACAUG
1094	UUUAAUU	CUGAUGAGGCCGAAAGGCCGAA	AAAACAU
1095	CUUUAUU	CUGAUGAGGCCGAAAGGCCGAA	AAAAACA
1098	ACUCUUU	CUGAUGAGGCCGAAAGGCCGAA	AUUAAAA
1099	UACUCUU	CUGAUGAGGCCGAAAGGCCGAA	AAUUAAA

Table BVIII: Mouse B7-2 Hammerhead Ribozyme Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
47	AcGGACU u GaACAac	194	cuUAuUU C aAUGGgA
47	aCggACU u gaAcAAC	208	acUGCaU a UCUGCcG
66	CUccUgU a gAcGUGU	210	UGCaUaU C UGCcGug
66	CUCcUgU A gAcGUGu	223	UGCCcAU U UaCAAAg
74	gAcGUGU u CcagAAc	223	UGCcCAU u UAaAaAg
83	CaGaACU U aCggaAG	224	GCCcCAU U aCAAAgg
134	caAuCcU U aUCUUUG	225	ccCAUUU a CAAAggc
134	CaauccU U AUCUUug	225	CccaUUU a cAAAgGc
134	caAUCcU u AuCUUUG	242	AAaACAU a agCcUGa
134	CAaUccU U AUCuuUG	260	AGCUgGU A GUUUUUU
134	CAAUcCU U AUcuuUG	260	aGCUgGU a gUAUuuU
135	aAuCcUU a UCuuUGU	263	UgGUAGU A UUUUGGC
135	aAuCcUU a UCuuUgu	263	UGgUaGU a UuuUGgC
135	AaUccUU A UcuuUGU	265	GUAGUAU U UUGGCAG
135	aAUccUU a UCuuUgU	265	guAGUAU u UuGGCaG
137	uCcUUaU C UUUGUGA	266	UAGUAUU U UGGCAGG
137	UccUUaU c UuUGUGA	266	uAGUaUU U UGgcAgG
137	UCCuUAU c uuUGUGA	266	UAgUauU u UGGcAgg
139	cUUaUCU U UGUGAca	267	AGUAUUU U GGCAGGA
140	UUaUCUU U GUGAcaG	267	AGUaUUU U GgcAgGA
140	UUaUcuU U guGACAG	286	cAAAaGU U GGUUCUG
149	UGAcaGU c UUGCUGa	286	CAAAaGU U GgUUCuG
151	AcAGucU U GCUgaUC	290	AgUUGGU U CUGuAcG
151	AcaGuCU U gCUGaUC	291	gUUGGUU C UGUAcGA
158	UgCuGAU c UcAGaUg	295	GUUCuGU a CgAGcAc
158	UgCUGaU C UCaGaUG	304	GAGcacU A uUUgGGC
158	UGcUGaU c uCagaUg	307	cacUAUU u GGgCACA
158	UgCugAU c UCagAUg	323	AGAAAcU U GAuAGUG
160	CUGaUCU C aGaUGCU	343	gCCAAGU A ccUGGGC
160	cUGaUcU c AgAuGcU	343	gCCAagU a CCTgGGc
170	AUGcuGU u UcCgUgG	361	ACgAGcU U UGAcagG
171	UGCUGuU u CcgUGgA	381	cUGgACT c UacGACU
172	gCUgUuU C cgUgGAG	383	GgACUcU A CGACUcU
189	GcaaGcU u AUUUCaA	383	GGACuCU a cGaCUuC
189	gCAAGCU U AUUUCAA	389	uAcGacU u CaCAaUG
189	GCaaGCU u AuUUCaA	389	UacGACU U CACAAUG
190	CAAGCUU A UUUCAAU	390	acGACUU C ACAAUgU
190	CaAgcUU a uUUcaAU	390	ACgAcUU c acAAUgU
192	AGCUUAU U UCAAUGg	398	ACAAUGU U CagauCA
192	aGCUUaU u UCAAUGg	398	ACAAUGU U CAGAUCA
193	GCUUAUU U CAAUGgG	398	ACaAuGU U cagAUCA
193	GcuUAuU U CaAUGGg	399	CAaUGUU C AgauCAA
194	CUUAUUU C AAUGgGA	399	CAAUGUU C AGAUCAA

399	CaAuGUU c agAUCAa	658	CAGAUUAU c AcaagAu
399	caAUGUU c aGAUCAA	658	CagauAU C ACAAgAu
399	CAaUguU c aGAUCaA	658	CAGauAU C aCAAGAU
399	cAAuGuU C aGAUcAA	658	CaGAUaU c ACaAGau
399	CAaugUU c agAUcAA	666	aCAAGAU A AUGUCAC
404	UUCAGAU C AAGGACA	666	ACAagaU a AUGucAC
404	UucAGaU c aAGGACa	671	AUAaUGU C ACAGaAc
418	aUGgGCU c GUAugAU	671	aUAAUgU c ACAGAAc
418	AuGGGCU c GUAugAu	671	AUAAUGU C ACAGAAc
418	AUggGCU c GUaUGaU	682	gAACUgU u cAGUAUc
421	ggCUCgU a UGAuugU	683	aAcUGuU c aGuAUcU
421	ggCUCgU A UgaUUGU	683	AACUGuU c agUaUcU
429	UgaUUGU u UuAUaCA	691	aguaUcU C CAaCAGC
429	UGAUuGU u UUAUaCA	691	agUAUCU c CAaCagc
431	AuUgUuU u AUAcAAa	691	aGUAucU C CAACAGc
431	AUuGUuU U AUaCAaA	701	aCaGCcU c UcUCUuU
432	UuGUuUU A UaCAaAA	701	acagCCU c UCUCUuU
432	UuGUUUU a UacaaAA	703	AGCcUcU C UcUUUCA
432	uUGUUUU a uAcaAAA	703	aGCcUcU c UCUnuca
461	gAUcaAU u AUCCucC	707	UcUCUcU U UCAUUCc
462	AucaAUU a uCcUCCA	707	UcUCUcU u UcaUUCc
464	CAauUaU c CUcCaAc	708	cUCUcUU U CAUUCcC
467	uUAUCcU C CAaCAGa	709	UCUcUUU C AUUCcCg
467	UUauCcU C CAaCAGA	709	UCUcUUU c auuCCcG
467	UUaUccU c cAACAGA	709	UCUcUUU c AUUCcCg
467	UuAuCCU C CaaCAGA	712	CUUUCaU U CcCgGaU
490	GAACUGU C AGUGaUc	712	cuuUCAU U cCCgGAU
497	CAGUGaU c GCcAACU	712	CuUucAU u CcCGGaU
505	GCcAACU U CAGUGAA	712	cUUUCAU U cCCgGAU
506	CcAACUU C AGUGAAC	712	CUUUCaU u ccCggaU
506	CCAaCUU C aGUGaaC	713	uuUCAU U cCCgGAUg
521	CUGAAAU A aaACugg	713	UUUCAU C cCCgGAUG
531	ACUGgcU c AgAaUgU	732	GuGgcAU a UGACCgU
539	agaaUGU A ACAGGAa	732	GuGgcAU A UGACCgU
550	GgAaAuU c uGGCAuA	740	UGACCgU u gUgUGUg
550	ggAAaUU C UggcAUa	749	UgUGUgU U CUGGAAA
557	cuggCAU A AAUUUGA	749	uGuGUGU U cUggAAA
561	CAUAAAU U UGACCUG	750	gUGUGUU C UGGAAAC
562	AUAAAUU U GACCUGC	750	GuGUGUU c UggAAAc
576	CaCgUCU A agCAaGG	773	ugAAGaU U UcCUcCa
585	gCAaGGU c ACCCgaA	778	aUUUcCU c caAACCU
597	gaAACTU A AGAAGAU	788	AAcCTCU C AAuuuCA
607	AaGaUgU a uUuUCUg	798	UUUCaCU c aAGAGuU
611	UGUaUUU u cUgAuAa	805	CAagAGU U UccAUcU
625	AcuAAUU C AACUAau	805	CAagAGU U uccAUcU
630	UUCAACU A auGAGUA	806	AAgAGU U ccAUcUc
630	UUcAAcU A AuGAGUA	811	UUUCCA U c ucCUcaa
637	AauGAGU A UGgUGaU	811	uUUCaU c UcCUcaA
656	uGCAGaU a UcAcAAG	813	uCCAUCU c CUcaAac

836	aGgAGAU U	acAGCUU
836	aggaGAU U	ACAGCUu
837	GgAGAUU a	cAGCUUc
848	CUUCAGU u	AcugUGg
860	UGGCCcU C	CUcCUug
860	UggCCcU c	CUCcuUg
878	ugCUGCU C	AUCauUg
951	GCGGgaU a	GuAACgC
974	AgaCuAU c	aACCUgA
989	aGgaAcU U	GaACCCc
1006	auUgCUU c	aGCAAAa
1055	AAAgAGU u	aaAAaUU
1056	AaGAgUU a	aaAAuUG
1062	uAAAAAU u	gcUuUgC
1092	CAgaGUU u	CuCAGAA
1095	aGUUUcU c	AgAaUUC
1101	UCAgAAU u	caaAaAU
1101	ucAGAAU U	CAAAaAU
1101	UcAgAaU U	CaAAaAu
1111	aAaAUGU U	cUcAgcU
1112	AaAUGUU c	UcAgcUg
1128	UUgGAaU u	cuACAGU
1128	UUGGAaU u	CuaCaGU
1131	GAAuUCU a	cAGuUgA
1131	GAauUCU a	CaguUgA
1141	GuUGAAU a	aUuAAag
1144	gaaUAAU U	AAAGAc
1145	AAuAaUU a	aAgACA

Table BIX: Mouse B7-2 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequences
47	GUUGUUC CUGAUGAGGCCGAAAGGCCGAA AGUCCGU
47	GUUGUUC CUGAUGAGGCCGAAAGGCCGAA AGUCCGU
66	ACACGUC CUGAUGAGGCCGAAAGGCCGAA ACAGGAG
66	ACACGUC CUGAUGAGGCCGAAAGGCCGAA ACAGGAG
74	GUUCUGG CUGAUGAGGCCGAAAGGCCGAA ACACGUC
83	CUUCCGU CUGAUGAGGCCGAAAGGCCGAA AGUUCUG
134	CAAAGAU CUGAUGAGGCCGAAAGGCCGAA AGGAUUG
134	CAAAGAU CUGAUGAGGCCGAAAGGCCGAA AGGAUUG
134	CAAAGAU CUGAUGAGGCCGAAAGGCCGAA AGGAUUG
134	CAAAGAU CUGAUGAGGCCGAAAGGCCGAA AGGAUUG
134	CAAAGAU CUGAUGAGGCCGAAAGGCCGAA AGGAUUG
135	ACAAAGA CUGAUGAGGCCGAAAGGCCGAA AAGGAUU
135	ACAAAGA CUGAUGAGGCCGAAAGGCCGAA AAGGAUU
135	ACAAAGA CUGAUGAGGCCGAAAGGCCGAA AAGGAUU
135	ACAAAGA CUGAUGAGGCCGAAAGGCCGAA AAGGAUU
137	UCACAAA CUGAUGAGGCCGAAAGGCCGAA AUAAGGA
137	UCACAAA CUGAUGAGGCCGAAAGGCCGAA AUAAGGA
137	UCACAAA CUGAUGAGGCCGAAAGGCCGAA AUAAGGA
139	UGUCACA CUGAUGAGGCCGAAAGGCCGAA AGAUAAG
140	CUGUCAC CUGAUGAGGCCGAAAGGCCGAA AAGAUAA
140	CUGUCAC CUGAUGAGGCCGAAAGGCCGAA AAGAUAA
149	UCAGCAA CUGAUGAGGCCGAAAGGCCGAA ACUGUCA
151	GAUCAGC CUGAUGAGGCCGAAAGGCCGAA AGACUGU
151	GAUCAGC CUGAUGAGGCCGAAAGGCCGAA AGACUGU
158	CAUCUGA CUGAUGAGGCCGAAAGGCCGAA AUCAGCA
158	CAUCUGA CUGAUGAGGCCGAAAGGCCGAA AUCAGCA
158	CAUCUGA CUGAUGAGGCCGAAAGGCCGAA AUCAGCA
158	CAUCUGA CUGAUGAGGCCGAAAGGCCGAA AUCAGCA
160	AGCAUCU CUGAUGAGGCCGAAAGGCCGAA AGAUCAG
160	AGCAUCU CUGAUGAGGCCGAAAGGCCGAA AGAUCAG
170	CCACGGA CUGAUGAGGCCGAAAGGCCGAA ACAGCAU
171	UCCACGG CUGAUGAGGCCGAAAGGCCGAA AACAGCA
172	CUCCACG CUGAUGAGGCCGAAAGGCCGAA AAACAGC
189	UUGAAAU CUGAUGAGGCCGAAAGGCCGAA AGCUUGC
189	UUGAAAU CUGAUGAGGCCGAAAGGCCGAA AGCUUGC
189	UUGAAAU CUGAUGAGGCCGAAAGGCCGAA AGCUUGC
190	AUUGAAA CUGAUGAGGCCGAAAGGCCGAA AAGCUUG
190	AUUGAAA CUGAUGAGGCCGAAAGGCCGAA AAGCUUG
192	CCAUUGA CUGAUGAGGCCGAAAGGCCGAA AUAAGCU
192	CCAUUGA CUGAUGAGGCCGAAAGGCCGAA AUAAGCU
193	CCCAUUG CUGAUGAGGCCGAAAGGCCGAA AAUAAGC
193	CCCAUUG CUGAUGAGGCCGAAAGGCCGAA AAUAAGC
194	UCCCAUU CUGAUGAGGCCGAAAGGCCGAA AAUAAG

194 UCCCAUU CUGAUGAGGCCGAAAGGCCGAA AAAUAAG
208 CGGCAGA CUGAUGAGGCCGAAAGGCCGAA AUGCAGU
210 CACGCA CUGAUGAGGCCGAAAGGCCGAA AUAUGCA
223 CUUUGUA CUGAUGAGGCCGAAAGGCCGAA AUGGGCA
223 CUUUGUA CUGAUGAGGCCGAAAGGCCGAA AUGGGCA
224 CCUUGU CUGAUGAGGCCGAAAGGCCGAA AAUGGGC
225 GCUUG CUGAUGAGGCCGAAAGGCCGAA AAAUGGG
225 GCUUG CUGAUGAGGCCGAAAGGCCGAA AAAUGGG
242 UCAGGCU CUGAUGAGGCCGAAAGGCCGAA AUGUUUU
260 AAAAUAC CUGAUGAGGCCGAAAGGCCGAA ACCAGCU
260 AAAAUAC CUGAUGAGGCCGAAAGGCCGAA ACCAGCU
263 GCCAAA CUGAUGAGGCCGAAAGGCCGAA ACTUCCA
263 GCCAAA CUGAUGAGGCCGAAAGGCCGAA ACTUCCA
265 CUGCCA CUGAUGAGGCCGAAAGGCCGAA AUACUAC
265 CUGCCA CUGAUGAGGCCGAAAGGCCGAA AUACUAC
266 CCUGCCA CUGAUGAGGCCGAAAGGCCGAA AAUACUA
266 CCUGCCA CUGAUGAGGCCGAAAGGCCGAA AAUACUA
266 CCUGCCA CUGAUGAGGCCGAAAGGCCGAA AAUACUA
267 UCCUGCC CUGAUGAGGCCGAAAGGCCGAA AAAUACU
267 UCCUGCC CUGAUGAGGCCGAAAGGCCGAA AAAUACU
286 CAGAAC CUGAUGAGGCCGAAAGGCCGAA ACUUUUG
286 CAGAAC CUGAUGAGGCCGAAAGGCCGAA ACUUUUG
290 CGUACAG CUGAUGAGGCCGAAAGGCCGAA ACCAACTU
291 UCGUACA CUGAUGAGGCCGAAAGGCCGAA AACCAAC
295 GUGCUCG CUGAUGAGGCCGAAAGGCCGAA ACAGAAC
304 GCCCAA CUGAUGAGGCCGAAAGGCCGAA AGUGTUC
307 UGUGCCC CUGAUGAGGCCGAAAGGCCGAA AAUAGUG
323 CACUauc CUGAUGAGGCCGAAAGGCCGAA AGUUUCU
343 GCCCAGG CUGAUGAGGCCGAAAGGCCGAA ACTUGGC
343 GCCCAGG CUGAUGAGGCCGAAAGGCCGAA ACTUGGC
361 CCUGUCA CUGAUGAGGCCGAAAGGCCGAA AGUCUGU
381 AGUCGUA CUGAUGAGGCCGAAAGGCCGAA AGUCCAG
383 GAAGUCG CUGAUGAGGCCGAAAGGCCGAA AGAGUCC
383 GAAGUCG CUGAUGAGGCCGAAAGGCCGAA AGAGUCC
389 CAUUGUG CUGAUGAGGCCGAAAGGCCGAA AGUCGUA
389 CAUUGUG CUGAUGAGGCCGAAAGGCCGAA AGUCGUA
390 ACAUUGU CUGAUGAGGCCGAAAGGCCGAA AAGUCGU
390 ACAUUGU CUGAUGAGGCCGAAAGGCCGAA AAGUCGU
398 UGAUCUG CUGAUGAGGCCGAAAGGCCGAA ACAUUGU
398 UGAUCUG CUGAUGAGGCCGAAAGGCCGAA ACAUUGU
398 UGAUCUG CUGAUGAGGCCGAAAGGCCGAA ACAUUGU
399 UUGAUCU CUGAUGAGGCCGAAAGGCCGAA AACAUUG
399 UUGAUCU CUGAUGAGGCCGAAAGGCCGAA AACAUUG
399 UUGAUCU CUGAUGAGGCCGAAAGGCCGAA AACAUUG
399 UUGAUCU CUGAUGAGGCCGAAAGGCCGAA AACAUUG
399 UUGAUCU CUGAUGAGGCCGAAAGGCCGAA AACAUUG
399 UUGAUCU CUGAUGAGGCCGAAAGGCCGAA AACAUUG
404 UGUCCUU CUGAUGAGGCCGAAAGGCCGAA AUCUGAA

404 UGUCCU CUGAUGAGGCCGAAAGGCCGAA AUCUGAA
418 AUCAUAC CUGAUGAGGCCGAAAGGCCGAA AGCCCAU
418 AUCAUAC CUGAUGAGGCCGAAAGGCCGAA AGCCCAU
418 AUCAUAC CUGAUGAGGCCGAAAGGCCGAA AGCCCAU
421 ACAAUCA CUGAUGAGGCCGAAAGGCCGAA ACGAGCC
421 ACAAUCA CUGAUGAGGCCGAAAGGCCGAA ACGAGCC
429 UGUUAUA CUGAUGAGGCCGAAAGGCCGAA ACAAUCA
429 UGUUAUA CUGAUGAGGCCGAAAGGCCGAA ACAAUCA
431 UUUGUAU CUGAUGAGGCCGAAAGGCCGAA AAACAAU
431 UUUGUAU CUGAUGAGGCCGAAAGGCCGAA AAACAAU
432 UUUGUA CUGAUGAGGCCGAAAGGCCGAA AAAACAA
432 UUUGUA CUGAUGAGGCCGAAAGGCCGAA AAAACAA
432 UUUGUA CUGAUGAGGCCGAAAGGCCGAA AAAACAA
461 GGAGGAU CUGAUGAGGCCGAAAGGCCGAA AUUGAUC
462 UGGAGGA CUGAUGAGGCCGAAAGGCCGAA AAUUGAU
464 GUUGGAG CUGAUGAGGCCGAAAGGCCGAA AUAUUGU
467 UCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGGAUAA
467 UCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGGAUAA
467 UCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGGAUAA
467 UCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGGAUAA
490 GAUCACU CUGAUGAGGCCGAAAGGCCGAA ACAGUUC
497 AGUUGGC CUGAUGAGGCCGAAAGGCCGAA AUCACUG
505 UUCACUG CUGAUGAGGCCGAAAGGCCGAA AGUUGGC
506 GUUCACU CUGAUGAGGCCGAAAGGCCGAA AAGUUGG
506 GUUCACU CUGAUGAGGCCGAAAGGCCGAA AAGUUGG
521 CCAGUUU CUGAUGAGGCCGAAAGGCCGAA AUUUCAG
531 ACAUUCU CUGAUGAGGCCGAAAGGCCGAA AGCCAGU
539 UUCUGU CUGAUGAGGCCGAAAGGCCGAA ACAUUCU
550 UAUCCA CUGAUGAGGCCGAAAGGCCGAA AAUUCC
550 UAUCCA CUGAUGAGGCCGAAAGGCCGAA AAUUCC
557 UCAAAU CUGAUGAGGCCGAAAGGCCGAA AUGCCAG
561 CAGGUA CUGAUGAGGCCGAAAGGCCGAA AUUUAUG
562 GCAGGUC CUGAUGAGGCCGAAAGGCCGAA AAUUAU
576 CCUGCU CUGAUGAGGCCGAAAGGCCGAA AGACGUG
585 UUCGGU CUGAUGAGGCCGAAAGGCCGAA ACCUUGC
597 AUCUUCU CUGAUGAGGCCGAAAGGCCGAA AGGUUUC
607 CAGAAA CUGAUGAGGCCGAAAGGCCGAA ACAUUCU
611 UUAUCAG CUGAUGAGGCCGAAAGGCCGAA AAUAACA
625 AUUAGUU CUGAUGAGGCCGAAAGGCCGAA AAUUGAU
630 UACUCAU CUGAUGAGGCCGAAAGGCCGAA AGUUGAA
630 UACUCAU CUGAUGAGGCCGAAAGGCCGAA AGUUGAA
637 AUCACCA CUGAUGAGGCCGAAAGGCCGAA ACUCAU
656 CUUGUA CUGAUGAGGCCGAAAGGCCGAA AUCUGA
658 AUCUUGU CUGAUGAGGCCGAAAGGCCGAA AUAUCUG
658 AUCUUGU CUGAUGAGGCCGAAAGGCCGAA AUAUCUG
658 AUCUUGU CUGAUGAGGCCGAAAGGCCGAA AUAUCUG
658 AUCUUGU CUGAUGAGGCCGAAAGGCCGAA AUAUCUG
666 GUGACAU CUGAUGAGGCCGAAAGGCCGAA AUCUUGU
666 GUGACAU CUGAUGAGGCCGAAAGGCCGAA AUCUUGU

671 GUUCUGU CUGAUGAGGCCGAAAGGCCGAA ACAUUUAU
671 GUUCUGU CUGAUGAGGCCGAAAGGCCGAA ACAUUUAU
671 GUUCUGU CUGAUGAGGCCGAAAGGCCGAA ACAUUUAU
682 GAUACUG CUGAUGAGGCCGAAAGGCCGAA ACAGUUC
683 AGAUACU CUGAUGAGGCCGAAAGGCCGAA AACAGUU
683 AGAUACU CUGAUGAGGCCGAAAGGCCGAA AACAGUU
691 GCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGAUACU
691 GCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGAUACU
691 GCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGAUACU
701 AAAGAGA CUGAUGAGGCCGAAAGGCCGAA AGGCUGU
701 AAAGAGA CUGAUGAGGCCGAAAGGCCGAA AGGCUGU
703 UGAAAGA CUGAUGAGGCCGAAAGGCCGAA AGAGGCU
703 UGAAAGA CUGAUGAGGCCGAAAGGCCGAA AGAGGCU
707 GGAUGA CUGAUGAGGCCGAAAGGCCGAA AGAGAGA
707 GGAUGA CUGAUGAGGCCGAAAGGCCGAA AGAGAGA
708 GGAUG CUGAUGAGGCCGAAAGGCCGAA AAGAGAG
709 CGGGAU CUGAUGAGGCCGAAAGGCCGAA AAAGAGA
709 CGGGAU CUGAUGAGGCCGAAAGGCCGAA AAAGAGA
709 CGGGAU CUGAUGAGGCCGAAAGGCCGAA AAAGAGA
712 AUCCGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAG
712 AUCCGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAG
712 AUCCGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAG
712 AUCCGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAG
712 AUCCGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAG
713 CAUCCG CUGAUGAGGCCGAAAGGCCGAA AAUGAAA
713 CAUCCG CUGAUGAGGCCGAAAGGCCGAA AAUGAAA
732 ACGGUCA CUGAUGAGGCCGAAAGGCCGAA AUGCCAC
732 ACGGUCA CUGAUGAGGCCGAAAGGCCGAA AUGCCAC
740 CACACAC CUGAUGAGGCCGAAAGGCCGAA ACGGUCA
749 UUUCCAG CUGAUGAGGCCGAAAGGCCGAA ACACACA
749 UUUCCAG CUGAUGAGGCCGAAAGGCCGAA ACACACA
750 GUUCCA CUGAUGAGGCCGAAAGGCCGAA AACACAC
750 GUUCCA CUGAUGAGGCCGAAAGGCCGAA AACACAC
773 UGGAGGA CUGAUGAGGCCGAAAGGCCGAA AUCUUCA
778 AGGUUUG CUGAUGAGGCCGAAAGGCCGAA AGGAAAU
788 UGAAAUU CUGAUGAGGCCGAAAGGCCGAA AGAGGUU
798 AACUCUU CUGAUGAGGCCGAAAGGCCGAA AGUGAAA
805 AGAUGGA CUGAUGAGGCCGAAAGGCCGAA ACUCUUG
805 AGAUGGA CUGAUGAGGCCGAAAGGCCGAA ACUCUUG
806 GAGAUGG CUGAUGAGGCCGAAAGGCCGAA AACUCUU
811 UUGAGGA CUGAUGAGGCCGAAAGGCCGAA AUGGAAA
811 UUGAGGA CUGAUGAGGCCGAAAGGCCGAA AUGGAAA
813 GUUUGAG CUGAUGAGGCCGAAAGGCCGAA AGAUGGA
836 AAGCUGU CUGAUGAGGCCGAAAGGCCGAA AUCUCCU
836 AAGCUGU CUGAUGAGGCCGAAAGGCCGAA AUCUCCU
837 GAAGCUG CUGAUGAGGCCGAAAGGCCGAA AAUCUCC
848 CCACAGU CUGAUGAGGCCGAAAGGCCGAA ACUGAAG
860 CAAGGAG CUGAUGAGGCCGAAAGGCCGAA AGGCCCCA
860 CAAGGAG CUGAUGAGGCCGAAAGGCCGAA AGGCCCCA

878	CAAUGAU	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCA
951	GCGUUC	CUGAUGAGGCCGAAAGGCCGAA	AUCCCGC
974	UCAGGUU	CUGAUGAGGCCGAAAGGCCGAA	AUAGUCU
989	GGGUUC	CUGAUGAGGCCGAAAGGCCGAA	AGUUCU
1006	UUUUGCU	CUGAUGAGGCCGAAAGGCCGAA	AAGCAAU
1055	AAUUUUU	CUGAUGAGGCCGAAAGGCCGAA	ACUCUUU
1056	CAAUUUU	CUGAUGAGGCCGAAAGGCCGAA	AACUCUU
1062	GCAAAGC	CUGAUGAGGCCGAAAGGCCGAA	AUUUUUA
1092	UUCUGAG	CUGAUGAGGCCGAAAGGCCGAA	AACUCUG
1095	GAAUUCU	CUGAUGAGGCCGAAAGGCCGAA	AGAAACU
1101	AUUUUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUCUGA
1101	AUUUUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUCUGA
1101	AUUUUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUCUGA
1111	AGCUGAG	CUGAUGAGGCCGAAAGGCCGAA	ACAUUUU
1112	CAGCUGA	CUGAUGAGGCCGAAAGGCCGAA	AACAUUU
1128	ACUGUAG	CUGAUGAGGCCGAAAGGCCGAA	AUUCCAA
1128	ACUGUAG	CUGAUGAGGCCGAAAGGCCGAA	AUUCCAA
1131	UCAACUG	CUGAUGAGGCCGAAAGGCCGAA	AGAAUUC
1131	UCAACUG	CUGAUGAGGCCGAAAGGCCGAA	AGAAUUC
1141	CUUUAUU	CUGAUGAGGCCGAAAGGCCGAA	AUUC AAC
1144	GUUCUUU	CUGAUGAGGCCGAAAGGCCGAA	AUUU UUC
1145	UGUUCUU	CUGAUGAGGCCGAAAGGCCGAA	AAUUAUU

Table BX: Human CD40 Hammerhead Ribozyme Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
9	CCUCGCU C GGGCGCC	440	UUGGGGU C AAGCAGA
24	CAGUGGU C CUGCCGC	449	AGCAGAU U GCUACAG
37	GCCUGGU C UCACCUC	453	GAUUGCU A CAGGGGU
39	CUGGUCU C ACCUCCG	461	CAGGGGU U UCUGAUA
44	CUCACCU C GCCAUGG	462	AGGGGUU U CUGAUAC
53	CCAUGGU U CGUCUGC	463	GGGGUUU C UGAUACC
54	CAUGGUU C GUCUGCC	468	UUCUGAU A CCAUCUG
57	GGUUCGU C UGCCUCU	473	AUACCAU C UGCGAGC
63	UCUGCCU C UGCAGUG	491	GCCCAGU C GGCUCUC
74	AGUGCGU C CUCUGGG	496	GUCCGCU U CUUCUCC
77	GCGUCCU C UGGGGCU	497	UCGGCUU C UUCUCCA
88	GGCUGCU U GCUGACC	499	GGCUUCU U CUCCA AU
101	CCGCUGU C CAUCCAG	500	GCUUCU C UCCAAUG
105	UGUCCAU C CAGAAC	502	UUCUUCU C CAAUGUG
139	AAACAGU A CCUAAUA	511	AAUGUGU C AUCUGCU
143	AGUACCU A AUAAACA	514	GUGUCAU C UGCUUUC
146	ACCUAAU A AACAGUC	519	AUCUGCU U UCGAAAA
153	AAACAGU C AGUGCUG	520	UCUGCUU U CGAAAAA
162	GUGCUGU U CUUUGUG	521	CUGCUUU C GAAAAAU
163	UGCUGUU C UUUGUGC	531	AAA AUGU C ACCCUUG
165	CUGUUCU U UGUGCCA	537	UCACCCU U GGACAAG
166	UGUUCUU U GUGCCAG	566	ACCUUGU U GUGCAAC
208	ACAGAGU U CACUGAA	599	CUGAUGU U GUCUGUG
209	CAGAGUU C ACUGAAA	602	AUGUUGU C UGUGGUC
227	AAUGCCU U CCUUGCG	609	CUGUGGU C CCCAGGA
228	AUGCCUU C CUUGCGG	618	CCAGGAU C GGCUGAG
231	CCUUCUU U GCGGUGA	641	UGGUGAU C CCAUCA
247	AGCGAAU U CCUAGAC	647	UCCCCAU C AUCUUCG
248	GCGAAUU C CUAGACA	650	CCAUCAU C UUCGGGA
251	AAUUCUU A GACACCU	652	AUCAUCU U CGGGAUC
292	CACAAAU A CUGCGAC	653	UCAUCUU C GGAUCC
308	CCAACCU A GGGCUUC	659	UCGGGAU C CUGUUUG
314	UAGGGCU U CGGGUCC	664	AUCCUGU U UGCCAUC
315	AGGGCUU C GGGUCCA	665	UCCUGUU U GCCAUCC
320	UUCGGGU C CAGCAGA	671	UUGCCAU C CUCUUGG
337	GGCACCU C AGAAACA	674	CCAUCUU C UUGGUGC
353	ACACCAU C UGCACCU	676	AUCCUCU U GGUGCUG
381	GCAUGU A CGAGUGA	686	UGCUGGU C UUAUCA
407	GCUGUGU C CUGCACC	688	CUGGUCU U UAUCAAA
418	CACCGCU C AUGCUUG	689	UGGUCUU U AUCAAAA
424	UCAUGCU C GCCCGGC	690	GGUCUUU A UCAAAAA
433	CCCGGCU U UGGGGUC	692	UCUUUAU C AAAAAGG
434	CCGGCUU U GGGGUCA	720	AACCAAU A AGGCCCC

755	AGGAGAU C AAUUUC
759	GAUCAAU U UUCCCGA
760	AUCAAUU U UCCCGAC
761	UCAAUUU U CCCGACG
762	CAAUUUU C CCGACGA
771	CGACGAU C UUCCUGG
773	ACGAUCU U CCUGGCU
774	CGAUCUU C CUGGCUC
781	CCUGGCU C CAACACU
795	UGCUGCU C CAGUGCA
810	GGAGACU U UACAUGG
811	GAGACTU U ACAUGGA
812	AGACTUU A CAUGGAU
830	AACCGGU C ACCCAGG
855	AGAGAGU C GCAUCUC
860	GU CGCAU C UCAGUGC
862	CGCAUCU C AGUGCAG
927	AGGCAGU U GGCCAGA
981	GGGAGCU A UGCCCAG
990	GCCCAGU C AGUGCCA

Table BXL: Human CD40 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequences
9	GGCGCCC CUGAUGAGGCCGAAAGGCCGAA AGCGAGG
24	GCGGCAG CUGAUGAGGCCGAAAGGCCGAA ACCACUG
37	GAGGUGA CUGAUGAGGCCGAAAGGCCGAA ACCAGGC
39	GCGAGGU CUGAUGAGGCCGAAAGGCCGAA AGACCAG
44	CCAUGGC CUGAUGAGGCCGAAAGGCCGAA AGGUGAG
53	GCAGACG CUGAUGAGGCCGAAAGGCCGAA ACCAUGG
54	GGCAGAC CUGAUGAGGCCGAAAGGCCGAA AACCAUG
57	AGAGGCA CUGAUGAGGCCGAAAGGCCGAA ACGAACC
63	CACUGCA CUGAUGAGGCCGAAAGGCCGAA AGGCAGA
74	CCCAGAG CUGAUGAGGCCGAAAGGCCGAA ACGCACU
77	AGCCCCA CUGAUGAGGCCGAAAGGCCGAA AGGACGC
88	GGUCAGC CUGAUGAGGCCGAAAGGCCGAA AGCAGCC
101	CUGGAUG CUGAUGAGGCCGAAAGGCCGAA ACAGCGG
105	GGUUCUG CUGAUGAGGCCGAAAGGCCGAA AUGGACA
139	UAUUAAG CUGAUGAGGCCGAAAGGCCGAA ACUGUUU
143	UGUUUAU CUGAUGAGGCCGAAAGGCCGAA AGGUACU
146	GACUGUU CUGAUGAGGCCGAAAGGCCGAA AUUAGGU
153	CAGCACU CUGAUGAGGCCGAAAGGCCGAA ACUGUUU
162	CACAAAG CUGAUGAGGCCGAAAGGCCGAA ACAGCAC
163	GCACAAA CUGAUGAGGCCGAAAGGCCGAA AACAGCA
165	UGGCACA CUGAUGAGGCCGAAAGGCCGAA AGAACAG
166	CUGGCAC CUGAUGAGGCCGAAAGGCCGAA AAGAACA
208	UUCAGUG CUGAUGAGGCCGAAAGGCCGAA ACUCUGU
209	UUUCAGU CUGAUGAGGCCGAAAGGCCGAA AACUCUG
227	CGCAAGG CUGAUGAGGCCGAAAGGCCGAA AGGCAUU
228	CCGCAAG CUGAUGAGGCCGAAAGGCCGAA AAGGCAU
231	UCACCGC CUGAUGAGGCCGAAAGGCCGAA AGGAAGG
247	GUCUAGG CUGAUGAGGCCGAAAGGCCGAA AUUCGCU
248	UGUCUAG CUGAUGAGGCCGAAAGGCCGAA AAUUCGC
251	AGGUGUC CUGAUGAGGCCGAAAGGCCGAA AGGAAUU
292	GUCGCAG CUGAUGAGGCCGAAAGGCCGAA AUUUGUG
308	GAAGCCC CUGAUGAGGCCGAAAGGCCGAA AGGUUGG
314	GGACCCG CUGAUGAGGCCGAAAGGCCGAA AGCCCTA
315	UGGACCC CUGAUGAGGCCGAAAGGCCGAA AAGCCCU
320	UCUGCTG CUGAUGAGGCCGAAAGGCCGAA ACCCGAA
337	UGUUUTU CUGAUGAGGCCGAAAGGCCGAA AGGUGCC
353	AGGUGCA CUGAUGAGGCCGAAAGGCCGAA AUGGUGU
381	UCACUCG CUGAUGAGGCCGAAAGGCCGAA ACAGUGC
407	GGUGCAG CUGAUGAGGCCGAAAGGCCGAA ACACAGC
418	CGAGCAU CUGAUGAGGCCGAAAGGCCGAA AGCGGUG
424	GCCGGGC CUGAUGAGGCCGAAAGGCCGAA AGCAUGA
433	GACCCCA CUGAUGAGGCCGAAAGGCCGAA AGCCGGG
434	UGACCCC CUGAUGAGGCCGAAAGGCCGAA AAGCCCG

440 UCUGCUU CUGAUGAGGCCGAAAGGCCGAA ACCCCAA
449 CUGUAGC CUGAUGAGGCCGAAAGGCCGAA AUCUGCU
453 ACCCCUG CUGAUGAGGCCGAAAGGCCGAA AGCAAUC
461 UAUCAGA CUGAUGAGGCCGAAAGGCCGAA ACCCCUG
462 GUUCAG CUGAUGAGGCCGAAAGGCCGAA AACCCCU
463 GGUAUCA CUGAUGAGGCCGAAAGGCCGAA AAACCCC
468 CAGAUGG CUGAUGAGGCCGAAAGGCCGAA AUCAGAA
473 GCUCGCA CUGAUGAGGCCGAAAGGCCGAA AUGGUUU
491 AGAAGCC CUGAUGAGGCCGAAAGGCCGAA ACUGGGC
496 GGAGAAG CUGAUGAGGCCGAAAGGCCGAA AGCCGAC
497 UGGAGAA CUGAUGAGGCCGAAAGGCCGAA AAGCCGA
499 AUUGGAG CUGAUGAGGCCGAAAGGCCGAA AGAAGCC
500 CAUUGGA CUGAUGAGGCCGAAAGGCCGAA AAGAAGC
502 CACAUUG CUGAUGAGGCCGAAAGGCCGAA AGAAGAA
511 AGCAGAU CUGAUGAGGCCGAAAGGCCGAA ACACAUU
514 GAAAGCA CUGAUGAGGCCGAAAGGCCGAA AUGACAC
519 UUUUCGA CUGAUGAGGCCGAAAGGCCGAA AGCAGAU
520 UUUUUCG CUGAUGAGGCCGAAAGGCCGAA AAGCAGA
521 AUUUUUC CUGAUGAGGCCGAAAGGCCGAA AAAGCAG
531 CAAGGGU CUGAUGAGGCCGAAAGGCCGAA ACAUUUU
537 CUUGUCC CUGAUGAGGCCGAAAGGCCGAA AGGGUGA
566 GUUGCAC CUGAUGAGGCCGAAAGGCCGAA ACCAGGU
599 CACAGAC CUGAUGAGGCCGAAAGGCCGAA ACAUCAG
602 GACCACA CUGAUGAGGCCGAAAGGCCGAA ACAACAU
609 UCCUGGG CUGAUGAGGCCGAAAGGCCGAA ACCACAG
618 CUCAGCC CUGAUGAGGCCGAAAGGCCGAA AUCCUGG
641 UGAUGGG CUGAUGAGGCCGAAAGGCCGAA AUCACCA
647 CGAAGAU CUGAUGAGGCCGAAAGGCCGAA AUGGGGA
650 UCCCGAA CUGAUGAGGCCGAAAGGCCGAA AUGAUGG
652 GAUCCCG CUGAUGAGGCCGAAAGGCCGAA AGAUGAU
653 GGAUCCC CUGAUGAGGCCGAAAGGCCGAA AAGAUGA
659 CAAACAG CUGAUGAGGCCGAAAGGCCGAA AUCCCGA
664 GAUGGCA CUGAUGAGGCCGAAAGGCCGAA ACAGGAU
665 GGAUGGC CUGAUGAGGCCGAAAGGCCGAA AACAGGA
671 CCAAGAG CUGAUGAGGCCGAAAGGCCGAA AUGGCAA
674 GCACCAA CUGAUGAGGCCGAAAGGCCGAA AGGAUGG
676 CAGCACC CUGAUGAGGCCGAAAGGCCGAA AGAGGAU
686 UGAUAAA CUGAUGAGGCCGAAAGGCCGAA ACCAGCA
688 UUUGAUA CUGAUGAGGCCGAAAGGCCGAA AGACCAG
689 UUUUGAU CUGAUGAGGCCGAAAGGCCGAA AAGACCA
690 UUUUUGA CUGAUGAGGCCGAAAGGCCGAA AAAGACC
692 CCTUUUU CUGAUGAGGCCGAAAGGCCGAA AUAAAGA
720 GGGGCTU CUGAUGAGGCCGAAAGGCCGAA AUUGGUU
755 GAAAAUU CUGAUGAGGCCGAAAGGCCGAA AUCUCCU
759 UCCGGAA CUGAUGAGGCCGAAAGGCCGAA AUUGAUC
760 GUCGGGA CUGAUGAGGCCGAAAGGCCGAA AAUUGAU
761 CGUCGGG CUGAUGAGGCCGAAAGGCCGAA AAAUUGA
762 UCGUCGG CUGAUGAGGCCGAAAGGCCGAA AAAAUUG
771 CCAGGAA CUGAUGAGGCCGAAAGGCCGAA AUCGUCC

773	AGCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGAUCGU
774	GAGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AAGAUCG
781	AGUGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCAGG
795	UGCACUG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCA
810	CCAUGUA	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCC
811	UCCAUGU	CUGAUGAGGCCGAAAGGCCGAA	AAGUCUC
812	AUCCAUG	CUGAUGAGGCCGAAAGGCCGAA	AAAGUCU
830	CCUGGGU	CUGAUGAGGCCGAAAGGCCGAA	ACCGGUU
855	GAGAUGC	CUGAUGAGGCCGAAAGGCCGAA	ACUCUCU
860	GCACUGA	CUGAUGAGGCCGAAAGGCCGAA	AUGCGAC
862	CUGCACU	CUGAUGAGGCCGAAAGGCCGAA	AGAUGC
927	UCUGGCC	CUGAUGAGGCCGAAAGGCCGAA	ACUGCCU
981	CUGGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCC
990	UGGCACU	CUGAUGAGGCCGAAAGGCCGAA	ACUGGGC

Table BXII: Mouse CD40 Hammerhead Ribozyme Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
18	GGUgucU u UGCCUCg	479	cAUCAcU U UUCgaaa
18	GGuguCU u UGCCucG	480	AUCacuU U UCGAAAA
24	UuUGCCU C gGCuGUG	481	UCacuUU U CGAAAAg
38	GCGcgCU a UGGGGCU	481	UCACuuU U cGAaAAG
62	CagcGGU c CaUCUag	492	AAAgUGU u AuCCcUG
62	CaGCgGU C CAUCuAG	560	CUaAUGU c aUCUGUG
66	gGUCCA U C uAGggCa	563	AUGUcaU C UGUGGUu
80	AGUGuGU u acgUGca	572	gUGGUuU a AagUCcC
80	AgUGUGU u AcgUGCa	572	GuGGUUU a aagUcCC
81	gUGugUU a CgUGCaG	577	UuAAagU c CCgGAuG
100	AAACAGU A CCUccac	620	UGGgcAU C CuCAUCA
126	CUGUgaU U UGUGCCA	626	UCCuCAU C AcCaUuu
127	UGUgaUU U GUGCCAG	632	uCAcCAU u UUCGGGg
170	CAGcUcU u gaGAAGA	632	UcaCCAU u uUCggGG
208	gGCGAAU U CUCAGcC	634	AcCAUuU U CGGGgUG
209	GCGAAUU C ucAGcCc	635	CCaUuuU c GgGGUGu
233	gGGAGAU u cgcUgUC	635	cCAUuuU C GGGgUgu
267	ACCCAAU c AAggGcu	635	CCAuuuU C ggGGUGu
267	AcCCAAU c AaGggCu	647	UGuUucU C UaUAUCA
275	aAGGGCU U CGGUua	649	uUucUCU a UAUCAAA
275	AaGGGCU U CgGgUua	651	ucUCUaU A UCAAAAA
276	AAGGCUU C GGGUuaA	653	UCUaUAU C AAAAAGG
281	UUCGGGU u aAGaAGg	735	gGAaGAU u aUCCcGG
281	UUcGGGU u AAGaAGg	759	cGCUUGU C CAGUGCA
314	ACACugU C UGuACCU	794	AgCCUGU C ACaCAGG
354	caAgGaU u GCgaGGC	794	AGcCUGU c acaCAGg
386	cCugUaU c CCUGGCU	819	AGAGAGU C GCAUCUC
394	CCUgGCU u uGGaGuu	824	GUCCGAU C UCAGUGC
394	CCuGGCU U UGGaGUu	826	CGCAUCU C AGUGCAG
395	CuGGCUU U GGaGUuA	876	cCCUGGU C UgAaCcC
429	caCUGAU A CCgUCUG	913	GGCUUGU U GCUGACC
434	AUACCgU C UGuCAuC	997	CUCAaCU u GCuuUuu
434	AUaCcGU c UGuCAUC	1003	uUGCUUU u uAAGgAU
441	CugUCAU C CcuGCCc	1003	uugCUUU u uAaGGAU
452	GCCCAGU C GGUUCU	1023	gaAAgCU c GGGCaUC
452	GCCCAGU C gGcuuCu	1048	CAGuGaU a UCUaccA
457	GUCCGCU U CUUCUCC	1052	gAUauCU a CCaaGuG
458	UCCGGCU C UUCUCCA	1081	CCAGagU u GuCUugc
460	GGCUUCU U CUCCA U	1084	gAGuUGU C uUGCUGC
461	GCUUCU C UCCA U	1086	gUugUCU U GcUGCgG
463	UUCUUCU C CAUcaG	1097	gCgGcGU U CACUGuA
472	AAuCAGU C AucaCUu	1098	CgGcGUU C ACUGuAA
472	AAUcagU c auCACuU	1118	cgUGGCU A CAGGaGU

1118	CgUGGCU a CAggAgU
1141	CgCaGCU u gUGCUCG
1164	aCCUGgU U GCCAUCa
1202	UGuaaUU a UUUaUaC
1220	gGcAuCU c AgAAACu
1220	GGCAuCU C AGAAACu
1228	aGAaACU c UAgcaGG
1253	AaCaGGU a GUGgAAu
1331	AGgAGcU U GCUgCcc
1362	uUuUGaU C CCugGGA
1373	gGGaCUU c AUgguAA
1373	GgGACUU c AugguAA
1413	uUGUCAU u UGaccUC
1443	GUaaUGU a CcccGUG
1470	CACaUAU c CUaaaAu
1492	GugGUGU a uUGuAga
1497	GuAuUGU A gaAaUuA
1508	auUauUU a aUCcGCC
1508	AUuAuUU a auCCGcC
1523	cuGGGuU u CUaccUG

Table BXIII: Mouse CD40 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequence
18	CGAGGCA CUGAUGAGGCCGAAAGGCCGAA AGACACC
18	CGAGGCA CUGAUGAGGCCGAAAGGCCGAA AGACACC
24	CACAGCC CUGAUGAGGCCGAAAGGCCGAA AGGCAAA
38	AGCCCCA CUGAUGAGGCCGAAAGGCCGAA AGCGCGC
62	CUAGAUG CUGAUGAGGCCGAAAGGCCGAA ACCGCTG
62	CUAGAUG CUGAUGAGGCCGAAAGGCCGAA ACCGCTG
66	UGCCCUA CUGAUGAGGCCGAAAGGCCGAA AUGGACC
80	UGCACGU CUGAUGAGGCCGAAAGGCCGAA ACACACU
80	UGCACGU CUGAUGAGGCCGAAAGGCCGAA ACACACU
81	CUGCACG CUGAUGAGGCCGAAAGGCCGAA AACACAC
100	GUGGAGG CUGAUGAGGCCGAAAGGCCGAA ACUGUUU
126	UGGCACA CUGAUGAGGCCGAAAGGCCGAA AUCACAG
127	CUGGCAC CUGAUGAGGCCGAAAGGCCGAA AAUCACA
170	UCUUCUC CUGAUGAGGCCGAAAGGCCGAA AGAGCTG
208	GGCUGAG CUGAUGAGGCCGAAAGGCCGAA AUUCGCC
209	GGGCUGA CUGAUGAGGCCGAAAGGCCGAA AAUUCGC
233	GACAGCG CUGAUGAGGCCGAAAGGCCGAA AUCUCCC
267	AGCCCUU CUGAUGAGGCCGAAAGGCCGAA AUUGGGU
267	AGCCCUU CUGAUGAGGCCGAAAGGCCGAA AUUGGGU
275	UAACCCG CUGAUGAGGCCGAAAGGCCGAA AGCCCUU
275	UAACCCG CUGAUGAGGCCGAAAGGCCGAA AGCCCUU
276	UUAACCC CUGAUGAGGCCGAAAGGCCGAA AAGCCCU
281	CCUUCUU CUGAUGAGGCCGAAAGGCCGAA ACCCGAA
281	CCUUCUU CUGAUGAGGCCGAAAGGCCGAA ACCCGAA
314	AGGUACA CUGAUGAGGCCGAAAGGCCGAA ACAGUGU
354	GCCUCGC CUGAUGAGGCCGAAAGGCCGAA AUCCUUG
386	AGCCAGG CUGAUGAGGCCGAAAGGCCGAA AUACAGG
394	AACUCCA CUGAUGAGGCCGAAAGGCCGAA AGCCAGG
394	AACUCCA CUGAUGAGGCCGAAAGGCCGAA AGCCAGG
395	UAACUCC CUGAUGAGGCCGAAAGGCCGAA AAGCCAG
429	CAGACGG CUGAUGAGGCCGAAAGGCCGAA AUCAGUG
434	GAUGACA CUGAUGAGGCCGAAAGGCCGAA ACGGUUU
434	GAUGACA CUGAUGAGGCCGAAAGGCCGAA ACGGUUU
441	GGGCAGG CUGAUGAGGCCGAAAGGCCGAA AUGACAG
452	AGAAGCC CUGAUGAGGCCGAAAGGCCGAA ACUGGGC
452	AGAAGCC CUGAUGAGGCCGAAAGGCCGAA ACUGGGC
457	GGAGAAG CUGAUGAGGCCGAAAGGCCGAA AGCCGAC
458	UGGAGAA CUGAUGAGGCCGAAAGGCCGAA AAGCCGA
460	AUUGGAG CUGAUGAGGCCGAAAGGCCGAA AGAAGCC
461	GAUUGGA CUGAUGAGGCCGAAAGGCCGAA AAGAAGC
463	CUGAUUG CUGAUGAGGCCGAAAGGCCGAA AGAAGAA
472	AAGUGAU CUGAUGAGGCCGAAAGGCCGAA ACUGAUU
472	AAGUGAU CUGAUGAGGCCGAAAGGCCGAA ACUGAUU

479 UUUUGAA CUGAUGAGGCCGAAAGGCCGAA AGUGAUG
480 UUUUCGA CUGAUGAGGCCGAAAGGCCGAA AAGUGAU
481 CUUUUCG CUGAUGAGGCCGAAAGGCCGAA AAAGUGA
481 CUUUUCG CUGAUGAGGCCGAAAGGCCGAA AAAGUGA
492 CAGGGAU CUGAUGAGGCCGAAAGGCCGAA ACACUUU
560 CACAGAU CUGAUGAGGCCGAAAGGCCGAA ACAUUAG
563 AACCACA CUGAUGAGGCCGAAAGGCCGAA AUGACAU
572 GGGACUU CUGAUGAGGCCGAAAGGCCGAA AAACCAC
572 GGGACUU CUGAUGAGGCCGAAAGGCCGAA AAACCAC
577 CAUCCGG CUGAUGAGGCCGAAAGGCCGAA ACUUUAA
620 UGAUGAG CUGAUGAGGCCGAAAGGCCGAA AUGCCCA
626 AAAUGGU CUGAUGAGGCCGAAAGGCCGAA AUGAGGA
632 CCCCGAA CUGAUGAGGCCGAAAGGCCGAA AUGGUGA
632 CCCCGAA CUGAUGAGGCCGAAAGGCCGAA AUGGUGA
634 CACCCCG CUGAUGAGGCCGAAAGGCCGAA AAAUGGU
635 ACACCCC CUGAUGAGGCCGAAAGGCCGAA AAAAUGG
635 ACACCCC CUGAUGAGGCCGAAAGGCCGAA AAAAUGG
635 ACACCCC CUGAUGAGGCCGAAAGGCCGAA AAAAUGG
647 UGAUUAU CUGAUGAGGCCGAAAGGCCGAA AGAAACA
649 UUGAUAU CUGAUGAGGCCGAAAGGCCGAA AGAGAAA
651 UUUUUGA CUGAUGAGGCCGAAAGGCCGAA AUAGAGA
653 CCUUUUU CUGAUGAGGCCGAAAGGCCGAA AUTAUAG
735 CCGGGAU CUGAUGAGGCCGAAAGGCCGAA AUCUUC
759 UGCACUG CUGAUGAGGCCGAAAGGCCGAA AGCAGCG
794 CCUGUGU CUGAUGAGGCCGAAAGGCCGAA ACAGGCU
794 CCUGUGU CUGAUGAGGCCGAAAGGCCGAA ACAGGCU
819 GAGAUGC CUGAUGAGGCCGAAAGGCCGAA ACUCUCU
824 GCACTGA CUGAUGAGGCCGAAAGGCCGAA AUGCGAC
826 CUGCACT CUGAUGAGGCCGAAAGGCCGAA AGAUGCG
876 GGGUUAU CUGAUGAGGCCGAAAGGCCGAA ACCAGGG
913 GGUCAGC CUGAUGAGGCCGAAAGGCCGAA AGCAGCC
997 AAAAAGC CUGAUGAGGCCGAAAGGCCGAA AGUUGAG
1003 AUCCUUA CUGAUGAGGCCGAAAGGCCGAA AAAGCAA
1003 AUCCUUA CUGAUGAGGCCGAAAGGCCGAA AAAGCAA
1023 GAUGCCC CUGAUGAGGCCGAAAGGCCGAA AGCUUUC
1048 UGGUAGA CUGAUGAGGCCGAAAGGCCGAA AUCACUG
1052 CACUUGG CUGAUGAGGCCGAAAGGCCGAA AGAUUUC
1081 GCAAGAC CUGAUGAGGCCGAAAGGCCGAA ACUCUGG
1084 GCAGCAA CUGAUGAGGCCGAAAGGCCGAA ACAACUC
1086 CCGCAGC CUGAUGAGGCCGAAAGGCCGAA AGACAAC
1097 UACAGUG CUGAUGAGGCCGAAAGGCCGAA ACGCCCG
1098 UUACAGU CUGAUGAGGCCGAAAGGCCGAA AACGCCG
1118 ACUCCUG CUGAUGAGGCCGAAAGGCCGAA AGCCACG
1118 ACUCCUG CUGAUGAGGCCGAAAGGCCGAA AGCCACG
1141 CGAGCAC CUGAUGAGGCCGAAAGGCCGAA AGCUGCG
1164 UGAUGGC CUGAUGAGGCCGAAAGGCCGAA ACCAGGU
1202 GUUAUAA CUGAUGAGGCCGAAAGGCCGAA AAUUAUA
1220 AGUUUCU CUGAUGAGGCCGAAAGGCCGAA AGAUGCC
1220 AGUUUCU CUGAUGAGGCCGAAAGGCCGAA AGAUGCC

1228	CCUGCUA	CUGAUGAGGCCGAAAGGCCGAA	AGUUUCU
1253	AUUCCAC	CUGAUGAGGCCGAAAGGCCGAA	ACCUGUU
1331	GGGCAGC	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCU
1362	UCCGAGG	CUGAUGAGGCCGAAAGGCCGAA	AUCAAAA
1373	UUACCAU	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCC
1373	UUACCAU	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCC
1413	GAGGUCA	CUGAUGAGGCCGAAAGGCCGAA	AUGACAA
1443	CACGGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAUUAC
1470	AUUUUAG	CUGAUGAGGCCGAAAGGCCGAA	AUAUGUG
1492	UCUACAA	CUGAUGAGGCCGAAAGGCCGAA	ACACCAC
1497	UAAUUUC	CUGAUGAGGCCGAAAGGCCGAA	ACAAUAC
1508	GGCGGAU	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAU
1508	GGCGGAU	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAU
1523	CAGGUAG	CUGAUGAGGCCGAAAGGCCGAA	AACCCAG

Table BXIV: Human B7 Hairpin Ribozyme and Target Sequence

nt. Position	Hairpin Ribozyme Sequence				Substrate
286	ACAGCAG	AGAA	GAUGAC	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	GUCAUCA GGC CUGCCUGU
291	GCAAAACA	AGAA	GGGCTUG	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	CAGCCCU GGC UGUUUUGC
295	AGUGCAA	AGAA	GGCAGG	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	CCUGCCU GUU UUGCACCU
437	GCACCAAG	AGAA	GAAAGA	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	UCUUUCA GCU CUUGGUGC
469	AACACCUG	AGAA	GAAGUG	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	CACUUUC GUU CAGGUGGU
518	GACCCAG	AGAA	GGUUG	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	CAACGCU GUC CUGUGGUC
540	AGCUCUUC	AGAA	GAAACA	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	UGUUUCU GUU GAAGAGCU
596	ACAUCAUA	AGAA	GCAACA	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	UGGUGCU GAC UAUGAUGU
644	CAAAGAUG	AGAA	GGUUCU	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	AGAACCG GAC CAUCUUUG
702	GUCCCCUC	AGAA	GAUGGG	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	CCCAUCU GAC GAGGGCAC
795	GUAGGGAA	AGAA	GCUUUG	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	CAAAAGCU GAC UUCCCUAC
819	AUUUCAA	AGAA	GAUUAU	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	UAUAUCU GAC UUUUGAAAU
939	UCUUGGA	AGAA	GUUGUG	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	CACAACA GUU UCCCAAGA
1012	ACACAUGA	AGAA	GUGGUU	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	AACACA GCU UCAUGUGU
1055	AGUUGAAG	AGAA	GAUUA	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	UGAAUCA GAC CUUCAACU
1103	ACGAUGGG	AGAA	GGUUAU	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	AUAACCU GCU CCACAUCCU
1159	GUAGGUCA	AGAA	GCAUAU	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	AUAUGCU GGC UGACCUAC
1163	ACCAUGAG	AGAA	GGCAGC	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	CCUGCCU GAC CUACUGCU
1171	UGGGCCAA	AGAA	GUAGGU	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	ACCUACU GCU UUGCCCCA
1356	GUGGGUAA	AGAA	GCUUAA	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	UUAAGCU GUU UUAACCCAC
1395	UCAGCUUA	AGAA	GAAAGA	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	UCUUUCA GAU UAAGCTUGA

Table BXV: Mouse B7 Hairpin Ribozyme and Target Sequence

nt. Position	Hairpin Ribozyme Sequence	Substrate
74	AGAAUUGG AGAA GAGUGU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	ACACUCU GUU CCAUUTUCU
114	AUCCACCC AGAA GAUGCU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	AGCAUCU GCC GGGUGGAU
154	AAUCCGAGA AGAA GAGAUG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	CAUCUCU GUU UCUCCGAUU
265	CCUGCAUC AGAA GACAAU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	AUUGUCA GUU GAUCCAGG
328	GACGAUC AGAA GCACAA ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	UUGUGCU GCU GAUUCGUC
331	AAAGACGA AGAA GCAGCA ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	UGCUGCU GAU UCGUCUUU
356	UCAUCAAC AGAA GNAGAC ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	GUUUAUA GGUUGAUA
373	CUGACUUG AGAA GUUGUU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	AACAACU GUC CAAAGUCAG
403	AAUCCGCA AGAA GCACAA ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	UAUUGCU GCC UUGCCGUU
481	CAUUGACA AGAA GCACCA ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	UGGUGCU GUC UGUCAUUG
529	CAUUAUAA AGAA GGUUCU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	AGAACCG GAC UUUUAUUG
584	GUGCCCGG AGAA GAAAGG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	CCUUUCA GAC CGGGGCAC
600	AACGACAC AGAA GUUUGU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	ACAUACA GCU GUGUGGUU
677	GUAGAGAA AGAA GCUUUG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	CAAAGCU GAC UUCUCUAC
741	GGAAAGCA AGAA GGUAAU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	AUUACCU GCU UUGCUUCC
1028	AUGAGGAC AGAA GUUAUU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	AAUAAUA GUC GUGGUCAU
1077	UCTUUCGA AGAA GCUUCU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	AGAAGCU GUU UCAGAAAGA
1116	GAAGGUAA AGAA GUUGUU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	AACAACA GCC UUAACUUC
1153	GGAAAGCG AGAA GUUCAG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	CUGAACA GAC CGUCUUC
1157	UUAAGGAA AGAA GUCUGU ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	ACAGACC GUC UUCUUAUA
1178	CCCACAUG AGAA GAGAAG ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	CUUCUCU GUC CAUGUGGG
1246	UCCGAAAG AGAA GCUAGC ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	GCUAGCU GAU CUUUCGGA
1523	CAGAAUAG AGAA GGCUC ACCAGAGAAACACACGUGUGGUACAUAUACCUUGUA	GAGGCUU GCC CUUUCUUG

Table BXVI: Human B7-2 Hairpin Ribozyme and Target Sequences

nt. Position	HP Ribozyme Sequences		Substrate	
25	GUUACAGC	AGAA GAGAG	ACACAGAGAAACACACACGUGUGGUA	CAUUAUACCUUGUA
28	CCUGUAC	AGAA GCAGAG	ACACAGAGAAACACACACGUGUGGUA	CAUUAUACCUUGUA
57	CCCCACUC	AGAA GUGUGU	ACACAGAGAAACACACACGUGUGGUA	CAUUAUACCUUGUA
162	CACACAG	AGAA GGAAGG	ACACAGAGAAACACACACGUGUGGUA	CAUUAUACCUUGUA
175	UUCAGAGG	AGAA GCACCA	ACACAGAGAAACACACACGUGUGGUA	CAUUAUACCUUGUA
214	CAUGGCAG	AGAA GCAGUC	ACACAGAGAAACACACACGUGUGGUA	CAUUAUACCUUGUA
380	CAGGGUCC	AGAA GUCCGA	ACACAGAGAAACACACACGUGUGGUA	CAUUAUACCUUGUA
408	UGUCCUUG	AGAA GAAGAU	ACACAGAGAAACACACACGUGUGGUA	CAUUAUACCUUGUA
480	CAGAAUUC	AGAA GTUGGA	ACACAGAGAAACACACACGUGUGGUA	CAUUAUACCUUGUA
575	UAUAGAUG	AGAA GGUCNA	ACACAGAGAAACACACACGUGUGGUA	CAUUAUACCUUGUA
710	AACAGACA	AGAA GAUGGA	ACACAGAGAAACACACACGUGUGGUA	CAUUAUACCUUGUA
718	GGGAUGA	AGAA GACAAG	ACACAGAGAAACACACACGUGUGGUA	CAUUAUACCUUGUA
730	CUCGUAC	AGAA GGGAAU	ACACAGAGAAACACACACGUGUGGUA	CAUUAUACCUUGUA
783	AAGAUAUA	AGAA GCGUCU	ACACAGAGAAACACACACGUGUGGUA	CAUUAUACCUUGUA
825	CUGGGGGA	AGAA GAGGGU	ACACAGAGAAACACACACGUGUGGUA	CAUUAUACCUUGUA
835	GGAAUGUG	AGAA GGGGGA	ACACAGAGAAACACACACGUGUGGUA	CAUUAUACCUUGUA
856	GGAAUGAC	AGAA GUAAUC	ACACAGAGAAACACACACGUGUGGUA	CAUUAUACCUUGUA
896	UAGAUAUA	AGAA GAAUAC	ACACAGAGAAACACACACGUGUGGUA	CAUUAUACCUUGUA
930	AGUUGCGA	AGAA GCUUCU	ACACAGAGAAACACACACGUGUGGUA	CAUUAUACCUUGUA
987	UUUUCUUG	AGAA GUUCAC	ACACAGAGAAACACACACGUGUGGUA	CAUUAUACCUUGUA
1027	UGGGCUUC	AGAA GAUCUU	ACACAGAGAAACACACACGUGUGGUA	CAUUAUACCUUGUA

Table EXVII: Mouse B7-2 Hairpin Ribozyme and Target Sequences

nt. Position	HP Ribozyme Sequences				Substrate
10	UCUUAAGC	AGAA	GCUUGC	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GCAAGCA GAC GCGUAAGA
42	UUGUCAA	AGAA	GUGCUG	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CAGCAAG GAC UUGAACAA
56	CUACAGGA	AGAA	GGUUGU	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	ACNACCA GAC UCCUGUAG
108	CAUGGUGC	AGAA	GSGGUC	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GACCCCA GAU GCACCAUG
146	AUCAGCAA	AGAA	GUCACA	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UGUGACA GUC UUGCTUGAU
154	CAUCUGAG	AGAA	GCAAGA	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UCUUGCU GAU CUCAGAGU
161	GAACAGC	AGAA	GAGAUC	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GAUCUCA GAU GCUGUUDC
167	UCCAAGGA	AGAA	GCAUCU	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	AGAUGCU GUU UCCUGUGA
211	AUGGCCAC	AGAA	GAUUG	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CAUAUCU GCC GUGCCCAU
400	UGUCCUUG	AGAA	GAACAU	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	AUGUDCA GAU CAAGGACA
679	AGAUACUG	AGAA	GUUCUG	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CAGAACU GUU CAGUAUCU
696	AAGAGAGA	AGAA	GUUGGA	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UCCAAACA GCC UCUCUCUU
716	CACACACC	AGAA	GCGAAU	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	AUUCCTCG GAU GGUGUGUG
737	ACACACAC	AGAA	GUCAUA	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UAUGACC GUU GUGUGUGU
839	GUAACTUGA	AGAA	GUAUUC	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GAUUAACA GCU UCAGUUUAC
874	CAUAGAUG	AGAA	GCAUCA	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UGAUGCU GCU CAUCAUUG
907	GCCUGCUA	AGAA	GAUUCG	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CGAAUCA GCC UAGCAGGC
929	AACUUAAGA	AGAA	GUGUUG	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CAACACA GCC UCUAAGUU
1115	UUCCAUUC	AGAA	GAGAAC	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	GUUCUCA GCU GAUUGGAA
1118	GAUUUCCA	AGAA	GCUCAG	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	CUACAGCU GAU UGGAUUC
1133	AUUUUUUC	AGAA	GUAGAA	ACCAGAGAAACACACGUGUGUGGUACAUAUACCTUGGUA	UUUCUACA GUU GAUUAUUU

Table BXVIII : Human CD40 Hairpin Ribozyme and Target Sequences

nt. Position	Hairpin Ribozyme Sequences										Substrate		
26	GACCAGGC	AGAA	GGACCA	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						UGGUCCU	GCC	GCCTUGGUC
29	UGAGACCA	AGAA	GCAGGA	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						UCCUGCC	GCC	UGGUUCUA
58	ACUGCAGA	AGAA	GACGAA	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						UUGGUU	GCC	UCUGCAGU
84	GGUCAGCA	AGAA	GCCCCA	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						UGGGGU	GCU	UGCUGACC
91	GGACAGCG	AGAA	GCAAGC	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						GTUUGU	GAC	CGCUGUCC
95	GGUUGGAC	AGAA	GUCAGC	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						GTUGACC	GCU	GUCCAUC
98	UCUGGAUG	AGAA	GCGGUC	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						GACCGU	GUC	CAUCCAGA
159	GCACAAG	AGAA	GCACUG	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						CAGUGU	GUU	CUUUGUC
414	CGAGCAUG	AGAA	GUGCAG	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						CUCCACC	GCU	CAUGCUUG
429	GACCCCAA	AGAA	GGGGGA	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						UCGCCCG	GCU	UUGGGGUC
445	CUGUAGCA	AGAA	GUUGA	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						UCAAGCA	GAU	UGCUACAG
483	GCGGACUG	AGAA	GGGCU	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						GAGCCU	GCC	CAGUCGGC
488	AAGAGCC	AGAA	GGGCG	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						CUGCCCA	GUC	GGUUCUU
492	GGAGAAGA	AGAA	GACTUG	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						CCAGUUG	GCU	UCUUCUCC
515	UUUUCGAA	AGAA	GAUGAC	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						GUCAUCU	GCU	UUCGAAAA
593	CAGACAAC	AGAA	GUCTUG	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						CAAGACU	GAU	GUUGUCUG
619	GGGCTUC	AGAA	GAUCCU	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						AGGAUGG	GCU	GAGAGCCC
661	GGUUGGCA	AGAA	GGAUCC	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						CGAUCCU	GUU	UGCCAUC
764	GGAGAUUC	AGAA	GGAAAA	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						UUUUCU	GAC	GAUCUUC
788	ACUGGAGC	AGAA	GUGUUG	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						CAACACU	GCU	GCUCCAGU
791	UGCACUGG	AGAA	GCAGUG	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						CACUGU	GCU	CCAGUGCA
924	CUUGGCC	AGAA	GCCUGU	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						ACAGGCA	GUU	GGCCAGAG
946	CTUGGAGC	AGAA	GCACCA	ACCAGAGAAACACAC	UUGUGGUACAUUACCTUGGUA						UGGUGCU	GCU	GTUGCAGG
949	ACCCUUGC	AGAA	GCAGCA	ACCAGAGAAACACACG	UUGUGGUACAUUACCTUGGUA						UGGUGCU	GCU	GCAGGGGU

Table BXIX: Mouse CD40 Hairpin Ribozyme and Substrate Sequences

nt. Position	HP Ribozyme Sequences			Substrate			
25	GCGGCAC	AGAA	GAGGCA	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	UGCCUCG	GCU	GUGCGCGC
45	UGUCAACA	AGAA	GCCCCA	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	UGGGGCU	GCU	UGUGGACA
59	CTUAGAUG	AGAA	GCTGUC	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	GACAGCG	GUC	CAUCUAGG
144	GCTUGUCA	AGAA	GCTUCC	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	GGAAGCC	GAC	UGACAAGC
164	UUCUCAAG	AGAA	GUGCAG	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	CUGACA	GCU	CUUGAGAA
212	UUCCACUG	AGAA	GAGNAU	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	AUUCUCA	GCC	CAGUGGAA
311	CAGGUACA	AGAA	GUGUCU	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	AGACACU	GUC	UGUACCUG
431	GGAUGACA	AGAA	GUAUCA	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	UGAUACC	GUC	UGUCAUCC
444	GCCGACUG	AGAA	GGAUG	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	CAUCCU	GCC	CAGUGCGC
449	AAGNAGCC	AGAA	GGGCAG	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	CUGCCCA	GUC	GGCUUUCU
453	GGAGRAGA	AGAA	GATUGG	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	CCAGUUG	GCU	UCUUCUCC
550	UGACAUUA	AGAA	GATUCG	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	CGAGUCA	GAC	UAAUGUCA
580	GGCTUGGC	AGAA	GGGACU	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	AGUCCCG	GAU	GCGAGCCC
592	GAUAGCC	AGAA	GGGCTC	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	GAGCCCU	GCU	GGUCAUUC
605	CCCAUCAC	AGAA	GGAAUG	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	CAUUCU	GUC	GUGAUUGG
701	UGCCGUG	AGAA	GCAGGG	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	CCUUGG	GCU	CGACGGCA
752	ACTUGAGC	AGAA	GUGTUA	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	UAAACC	GCU	GTUCCAGU
755	UGCAGUGG	AGAA	GCGGUG	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	CACCGCU	GCU	CCAGUGCA
787	GUGUGACA	AGAA	GACACC	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	GGUGUCA	GCC	UGUCACAC
890	CUUCNAA	AGAA	GUUCCA	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	UGGAACTU	GCU	UUUGGAGG
909	GTUCAGCA	AGAA	GCCAUC	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	GAUGGCU	GCU	UGCUGACC
916	UUCNAAAG	AGAA	GCAAGC	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	GCTUGCU	GAC	CUUUGNAA
975	UGACAGGG	AGAA	GCGAUG	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	CAUGCTU	GCC	CCCUUGCA
1137	CGAGCACA	AGAA	GCTUUCU	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	GCCCGCA	GCU	UGUGCUUG
1276	GUUUUAA	AGAA	GUUUCU	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	AGAAACA	GCU	UUUAAAAC
1334	CGGGUUG	AGAA	GCAAGC	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	GCTUGCTU	GCC	CAAACCCG
1352	GGAUCAAA	AGAA	GGUAAC	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	GUUACCU	GAU	UUUGAUCC
1512	AAACCCAG	AGAA	GAUUA	ACCAGAGAAACACACGUGUGGUACAUAUACCUGGUA	UUAAUCC	GCC	CUGGGUUU

Table CII: 2.5 μ mol RNA Synthesis Cycle

Reagent	Equivalents	Amount	Wait Time*
Phosphoramidites	6.5	163 μ L	2.5
S-Ethyl Tetrazole	23.8	238 μ L	2.5
Acetic Anhydride	100	233 μ L	5 sec
N-Methyl Imidazole	186	233 μ L	5 sec
TCA	83.2	1.73 mL	21 sec
Iodine	8.0	1.18 mL	45 sec
Acetonitrile	NA	6.67 mL	NA

* Wait time does not include contact time during delivery.

Table EVII: Deprotection of a 36 mer all ribo oligo using 70% ethylamine in aqueous. The data are as follows upon HPLC reprocessing:

Sample	OD's	% Full Length Product (FLP)	% frontside	%backside
MA 10'@65°	0.984	14.5073	71.6740	13.8186
MA 10'@65°	1.125	18.9269	67.8006	13.2725
EA rt 10'	0.925	16.5804	66.8186	16.6010
EA rt 10'	0.920	15.7421	67.5794	16.6785
EA rt 30'	0.971	17.4694	67.6782	14.8525
EA rt 30'	0.794	15.7587	69.8084	14.4329
EA 40° 10'	0.819	18.0827	66.4937	15.4236
EA 40° 10'	0.986	17.5763	66.7865	15.6372
EA 40° 15'	0.877	18.7963	67.0064	14.1999
EA 40° 15'	0.911	18.7808	70.7306	10.4885
EA 55° 10'	1.001	17.8810	66.4703	15.6487
EA 55° 10'	1.023	19.1069	68.6706	12.2225

Claims

1. An enzymatic nucleic acid having a hammerhead motif, wherein said nucleic acid comprises of at least five ribose residues, and wherein
5 said nucleic acid comprises a 2'-C-allyl modification at position No. 4 of said nucleic acid, and wherein said nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'- end modification.
2. The enzymatic nucleic acid of claim 1, wherein said nucleic acid
10 comprises a 3'-3' linked inverted ribose moiety at said 3' end.
3. An enzymatic nucleic acid having a hammerhead motif, wherein said nucleic acid comprises of at least five ribose residues, and wherein said nucleic acid comprises a 2'-amino modification at position No. 4 and/or at position No. 7 of said nucleic acid, wherein said nucleic acid
15 comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'-3' linked inverted ribose or thymidine moiety at its 3' end.
4. An enzymatic nucleic acid having a hammerhead motif, wherein said nucleic acid comprises of at least five ribose residues, and wherein
20 said nucleic acid comprises a non-nucleotide substitution at position No. 4 and/or at position No. 7 of said nucleic acid molecule, wherein said nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'-3' linked inverted ribose or thymidine moiety at its 3' end.
- 25 5. An enzymatic nucleic acid which cleaves target mRNA having a sequence selected from SEQ. ID. NOS. 34, 35, 57, 125, 126, 127, 128, 129, 140, 162, 170, 179, 188, 223, 224, 236, 245, 246, 256, 259, 260, and 281, wherein said nucleic acid comprises of at least five ribose residues, and wherein said nucleic acid comprises a 6-methyl
30 uridine substitution at position No. 4 and/or at position No. 7 of said nucleic acid molecule, wherein said nucleic acid comprises at least

ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'-3' linked inverted ribose or thymidine moiety at its 3' end.

- 5 6. The enzymatic nucleic acid which cleaves target mRNA having a sequence selected from SEQ. ID. NOS. 34, 35, 57, 125, 126, 127, 128, 129, 140, 162, 170, 179, 188, 223, 224, 236, 245, 246, 256, 259, 260, and 281, wherein said nucleic acid comprises of at least five ribose residues, wherein said nucleic acid comprises a 2'-C-allyl modification at position No. 4 of the said nucleic acid, wherein said
10 nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 2'-3' linked inverted ribose or thymidine moiety at its 3' end.
- 15 7. The enzymatic nucleic acid of any one of claims 1-6, wherein said nucleic acid comprises phosphorothioate linkages at least three of the seven 5' terminal nucleotides.
8. Nucleic acid molecule which blocks synthesis and/or expression of an mRNA encoding B7-1, B7-2, B7-3 and/or CD40.
9. The nucleic acid of claim 8, wherein said molecule is an enzymatic nucleic acid molecule.
- 20 10. The nucleic acid molecule of claim 9, wherein, the binding arms of said enzymatic nucleic acid contain sequences complementary to the nucleotide base sequences in any one of Tables BII, BIV, BVI, BVIII, BX, BXII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX.
- 25 11. The nucleic acid molecule of claims 9 or 10, wherein said nucleic acid molecule is in a hammerhead motif.
12. The enzymatic nucleic acid molecule of claim 9 or 10, wherein said nucleic acid molecule is in a hairpin, hepatitis Delta virus, group I intron, VS nucleic acid or RNaseP nucleic acid motif.

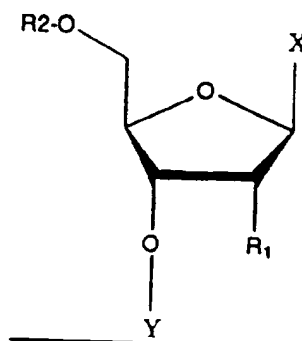
13. The enzymatic nucleic acid molecule of any of claims 9 or 10, wherein said ribozyme comprises between 12 and 100 bases complementary to the RNA of said region.
- 5 14. The enzymatic nucleic acid of claim 13, wherein said ribozyme comprises between 14 and 24 bases complementary to the RNA of said region.
- 15 15. Enzymatic nucleic acid molecule consisting essentially of any ribozyme sequence selected from those shown in Tables BIII, BV, BVI, BVII, BIX, BXI, BXIII, BXIV, BXV, BXVI, BXVII, BXVIII.
- 10 16. A mammalian cell including an enzymatic nucleic acid molecule of any of claims 8 or 9.
17. The cell of claim 16, wherein said cell is a human cell.
- 15 18. An expression vector comprising nucleic acid encoding the enzymatic nucleic acid molecule of any of claims 9 or 10, in a manner which allows expression and/or delivery of that enzymatic RNA molecule within a mammalian cell.
19. A mammalian cell including an expression vector of claim 18.
- 20 20. The cell of claim 19, wherein said cell is a human cell.
21. A method for treatment of a patient having a condition associated with the level of B7-1, B7-2, B7-3 and/or CD40, wherein the patient, tissue donor or population of corresponding cells is administered a therapeutically effective amount of an enzymatic nucleic acid molecule of claims 8, 9 or 10.
- 25 22. A method for treatment of a condition related to the level of B7-1, B7-2, B7-3 and/or CD40 activity by administering to a patient an expression vector of claim 21.
23. The method of claims 21 or 22, wherein said patient is a human.

24. A method for inducing tolerance in a recipient to alloantigen of a donor comprising treating antigen presenting cells from a donor with nucleic acid of claim 8 or 9, and infusion of said treated antigen presenting cells into said recipient.
- 5 25. A method for enhancing graft tolerance comprising contacting a nucleic acid of claims 8 or 9 with cells of said graft prior to transplantation.
26. A method for treatment of an autoimmune disease, comprising contacting an antigen presenting cell of a patient with a nucleic acid of claims 8 or 9.
- 10 27. The method of claim 26, wherein said cells are contacted *ex vivo* with said nucleic acid.
28. The method of claim 26, wherein said cells are contacted with autoantigen characteristic of said disease.
- 15 29. The method of claim 28, wherein said cells are reinfused into said patient.
- 20 30. Enzymatic nucleic acid having at least one modified base substitution, wherein said base substitution is selected from a group comprising pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyluracil, dihydrouracil, naphthyl, 6-methyl-uracil and aminophenyl.
31. The enzymatic nucleic acid of any of claim 30, wherein said nucleic acid has a hammerhead motif.
32. Mammalian cell comprising an enzymatic nucleic acid molecule of and of claims 30-31.
- 25 33. The enzymatic nucleic acid of claim 31, wherein said nucleic acid includes said modified base substitutions at position 4 or at position 7.
34. The ribozyme of claim 33, wherein said substitution is 6-methyl uracil.
35. The ribozyme of claim 33, wherein said substitution is pyridin-4-one.

36. The ribozyme of claim 33, wherein said substitution is phenyl.
37. The ribozyme of claim 33, wherein said substitution is pyridin-2-one.
38. The ribozyme of claim 33, wherein said substitution is pseudouracil.
39. The ribozyme of claim 33, wherein said substitution is 2, 4, 6-trimethoxy
5 benzene.
40. The ribozyme of claim 33, wherein said substitution is dihydrouracil.
41. The ribozyme of claim 33, wherein said substitution is 3-methyluracil.
42. The ribozyme of claim 33, wherein said substitution is naphthyl.
43. The ribozyme of claim 33, wherein said substitution is aminophenyl.
- 10 44. 2'-deoxy-2'-alkylnucleoside.
45. 2'-deoxy-2'-alkylnucleotide.
46. Oligonucleotide comprising one or more 2'-deoxy-2'-alkylnucleotides.
47. Enzymatic nucleic acid comprising a 2'-deoxy-2'-alkylnucleotide.
- 15 48. Method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, comprising the step of forming said enzymatic molecule with at least one nucleotide having at its 2'-position an alkyl group.
49. 2'-deoxy-2'-alkylnucleotide triphosphate.
- 20 50. Method for synthesis of a 2'-C-allyl derivative from a 5'-O-DMT-3'-O-TBDMS-base comprising the steps of:
- (a) phenoxyltriacarbonylation of 5'-O-DMT-3'-O-TBDMS-base to yield a thioester, replacing a 2' hydroxyl group with a phenoxythiocarbonyl group, and

(b) Heck acylation of said thioester to form a 2'-C-allyl derivative in which said 2'-phenoxythiocarbonyl group is replaced with said 2'-C-alkyl group to yield said 2'-C-allyl derivative.

51. A compound having the formula:



5

wherein, R1 represents 2'-O-alkylthioalkyl or 2'-C-alkylthioalkyl; X represents a base or H; Y represents a phosphorus-containing group; and R2 represents O, DMT or a phosphorus-containing group.

52. Oligonucleotide comprising one or more compounds of claim 51.

10 53. Enzymatic nucleic acid comprising a compound of claim 51.

54. The compound of claim 51, wherein said compound is in the form of a triphosphate.

55. Enzymatic nucleic acid of claim 53 wherein said nucleic acid is in a hammerhead motif.

15 56. Enzymatic nucleic acid of claim 53, wherein said nucleic acid is in a hairpin, hepatitis delta virus, group I intron, VS RNA or RNase P RNA motif.

20 57. Enzymatic nucleic acid of claim 55, wherein said hammerhead ribozyme has positions 4 and/or 7 substituted with 2'-O-methylthiomethyl.

58. Enzymatic nucleic acid of claim 55 or 57, wherein one monomer in stem II of said hammerhead is substituted with at least one 2'-O-methylthiomethyl.
59. Enzymatic nucleic acid of claim 55 or 56, wherein said nucleic acid is substituted at one or more positions with 2'-O-methylthiophenyl.
60. A mammalian cell comprising a compound of any one of the claims 51-59.
61. The cell of claim 60, wherein said cell is a human cell.
62. Method for producing an enzymatic nucleic acid molecule having activity to cleave an RNA or single-stranded DNA molecule, comprising the step of forming said enzymatic molecule with at least one position having at its 2'-position an 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl group.
64. Hammerhead ribozyme having a non-nucleotide in the catalytic core in a site selected from the group consisting of the normally occurring uracil at position 4 and 7.
65. Hammerhead ribozyme having a stem II and a loop II, wherein said loop II comprises a non-nucleotide.
66. Hammerhead ribozyme having a non-nucleotide at its 3' end.
67. A mammalian cell comprising an enzymatic nucleic acid molecule of any one of the claims 64-67.
68. The cell of claim 67, wherein said cell is a human cell.
69. Method of synthesis of abasic ribonucleoside mimetics described in figure 58.
70. A method for the deprotection of RNA comprising the step of providing aqueous ethylamine (EA) at between 25°C - 60°C for 5 to 30 minutes to remove any exocyclic amino protecting groups from protected RNA.

71. The method of claim 70 wherein, said ethylamine is provided at 40°C for 10 minutes.
72. The method of claim 70 wherein, said ethylamine is provided at 55°C for 10 minutes.
- 5 73. The method of claim 70, further comprising deprotection of RNA alkylsilyl protecting groups comprising, contacting said groups with anhydrous triethylamine•hydrogen fluoride (aHF•TEA) trimethylamine or diisopropylethylamine at between 60 °C-70 °C for 0.25-24 h.
- 10 74. The method of any one of claims 70-73 wherein, said RNA is an enzymatic RNA.
75. Method for synthesis of an enzymatic nucleic acid, comprising the steps of:
- 15 providing a 3' and a 5' portion of said enzymatic nucleic acid having independent chemically reactive groups at the 5' and 3' positions, respectively, under conditions in which a covalent bond is formed between said 3' and 5' portions by said chemically reactive groups, said bond being selected from the group consisting of, disulfide, morpholino, amide, ether, thioether, amine, a double bond, sulfonamide, ester, carbonate, hydrazone, said bond not being a
- 20 natural bond formed between a 5' phosphate group and a 3' hydroxyl group.
76. The method of claim 75, wherein said nucleic acid has a hammerhead motif and said 3' and 5' positions each have said chemically reactive groups in or immediately adjacent to the stem II region.
- 25 77. The method of claim 75, wherein one said chemically reactive group is $(CH_2)_nSH$ and the other chemically reactive group is $(CH_2)_nSH$, wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 30 78. The method of claim 75, wherein one said chemically reactive group is $(CH_2)_nNH_2$ and the other chemically reactive group is ribose, wherein

each n independently is an integer from 0 to 10 inclusive and may be the same or different.

- 5 79. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{NH}_2$ and the other chemically reactive group is COOH , wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 10 80. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{X}$ and the other chemically reactive group is $(\text{CH}_2)_n\text{OH}$ or $(\text{CH}_2)_n\text{SH}$; wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different; X is halogen.
- 15 81. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{NH}_2$ and the other chemically reactive group is CHO , wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 20 82. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{PPh}_3$ and the other chemically reactive group is CHO , wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 25 83. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{NH}_2$ and the other chemically reactive group is $(\text{CH}_2)_n\text{SO}_2\text{Cl}$, wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 30 84. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{OH}$ and the other chemically reactive group is COOH , wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
85. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{COH}$ and the other chemically reactive group is $(\text{CH}_2)_n\text{NH}_2$, wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.

86. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{COX}$ and the other chemically reactive group is $(\text{CH}_2)_n\text{OH}$, wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 5 87. The method of claim 78, wherein said conditions include provision of NaIO_4 in contact with said ribose, and subsequent provision of NaBH_4 or NaCNBH_3 .
88. The method of claim 79, wherein said conditions include provision of a coupling reagent.
- 10 89. A mixture comprising 5' and 3' portions of an enzymatic nucleic acid having a 3' and 5' chemically reactive group respectively selected from the group consisting of $(\text{CH}_2)_n\text{SH}$, $(\text{CH}_2)_n\text{NH}_2$, ribose, COOH , $(\text{CH}_2)_n\text{X}$, $(\text{CH}_2)_n\text{PPh}_3$, CHO , $(\text{CH}_2)_n\text{SO}_2\text{Cl}$, $(\text{CH}_2)_n\text{COX}$, $(\text{CH}_2)_n\text{X}$, $(\text{CH}_2)_n\text{OH}$, $(\text{CH}_2)_n\text{COH}$, and $(\text{CH}_2)_n\text{SH}$; wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different and X is halogen.
- 15
90. The method of claim 75, wherein one said chemically reactive group is linking group-SH and the other chemically reactive group is linking group-SH, wherein each linking group may be the same or different.
- 20 91. The method of claim 75, wherein one said chemically reactive group is linking group- NH_2 and the other chemically reactive group is ribose.
92. The method of claim 75, wherein one said chemically reactive group is linking group- NH_2 and the other chemically reactive group is COOH .
- 25 93. The method of claim 75, wherein one said chemically reactive group is linking group- X and the other chemically reactive group is linking group-OH or linking group-SH; wherein each linking group may be the same or different; X is halogen.
94. The method of claim 75, wherein one said chemically reactive group is linking group- NH_2 and the other chemically reactive group is CHO .

95. The method of claim 75, wherein one said chemically reactive group is linking group-PPh₃ and the other chemically reactive group is CHO.
96. The method of claim 75, wherein one said chemically reactive group is linking group-NH₂ and the other chemically reactive group is linking group-SO₂Cl, wherein each linking group may be the same or different.
97. The method of claim 75, wherein one said chemically reactive group is linking group-OH and the other chemically reactive group is COOH.
98. The method of claim 75, wherein one said chemically reactive group is linking group-COH and the other chemically reactive group is linking group-NH₂, wherein each linking group may be the same or different.
99. The method of claim 75, wherein one said chemically reactive group is linking group-COX and the other chemically reactive group is linking group-OH, wherein each linking group may be the same or different.
100. The method of claim 91, wherein said conditions include provision of NaIO₄ in contact with said ribose, and subsequent provision of NaBH₄ or NaCNBH₃.
101. The method of claim 100, wherein said conditions include provision of a coupling reagent.
102. A mixture comprising 5' and 3' portions of an enzymatic nucleic acid having a 3' and 5' chemically reactive group respectively selected from the group consisting of linking group-SH, linking group-NH₂, ribose, COOH, linking group-X, linking group-PPh₃, CHO, linking group-SO₂Cl, linking group-COX, linking group-X, linking group-OH, linking group-COH, and linking group-SH; wherein each linking group may be the same or different and X is halogen.
103. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said

stem comprises at least 8 base pairs wherein said molecule is transcribed by a RNA polymerase II promoter system.

- 5 104. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs, wherein said molecule is transcribed by a U6 small nuclear RNA promoter system.
- 10 105. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs, wherein said molecule is transcribed by an adenovirus VA1 RNA promoter system.
- 15 106. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs, wherein said molecule is a chimeric adenovirus VA1 RNA.
- 20 107. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs, wherein said intramolecular stem is separated from said desired RNA by a spacer sequence.
- 25 108. The RNA molecule of claim 107, wherein said spacer sequence is about 5-50 nucleotides.

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FIG. 2b.

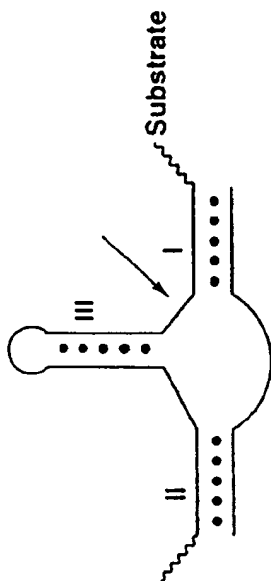


FIG. 2a.

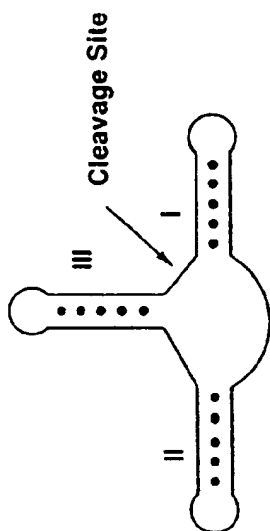


FIG. 2d.

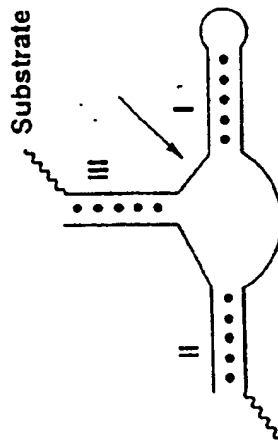
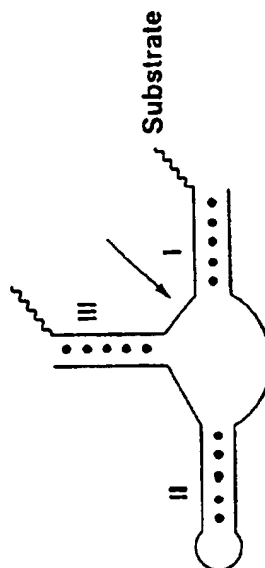


FIG. 2c.



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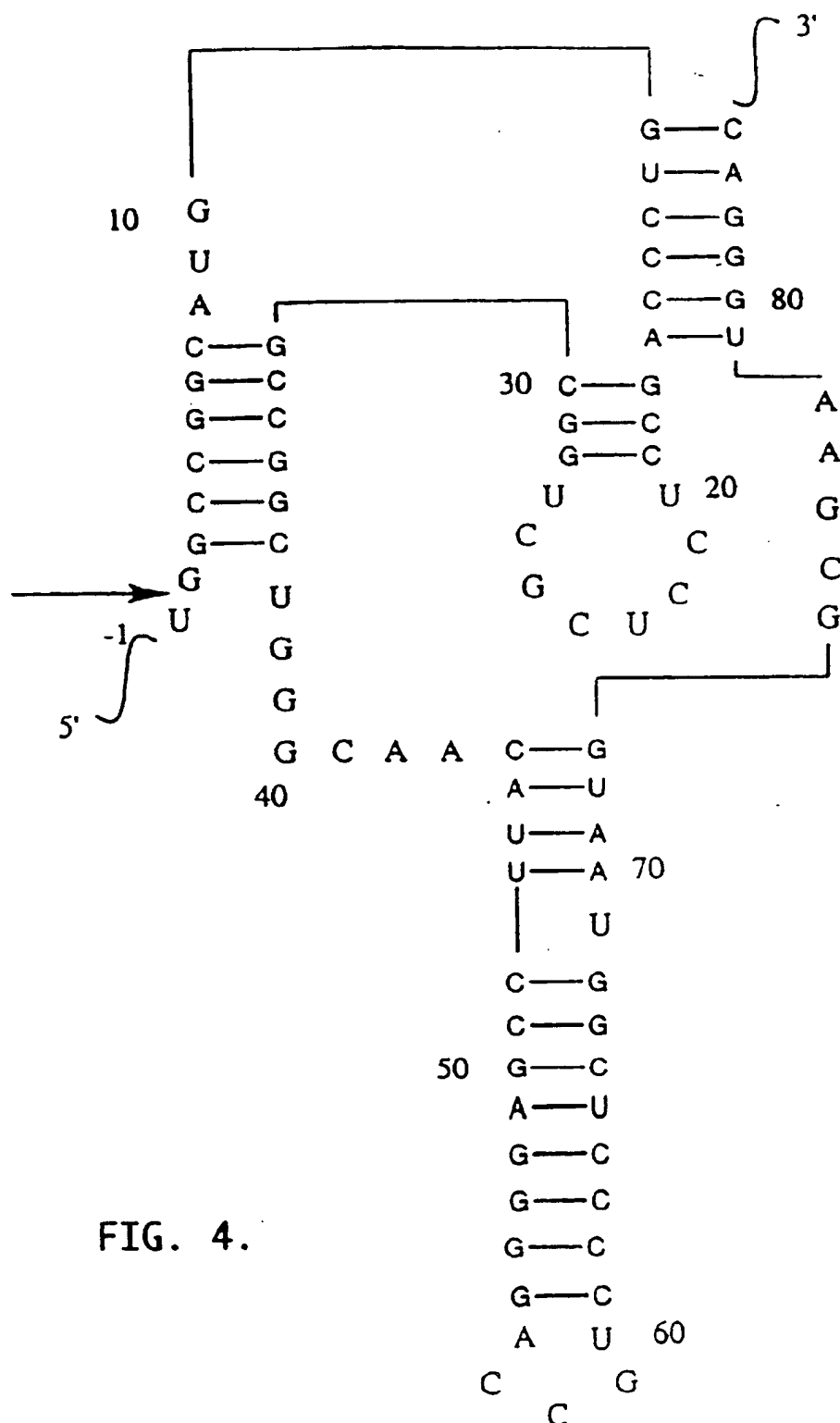
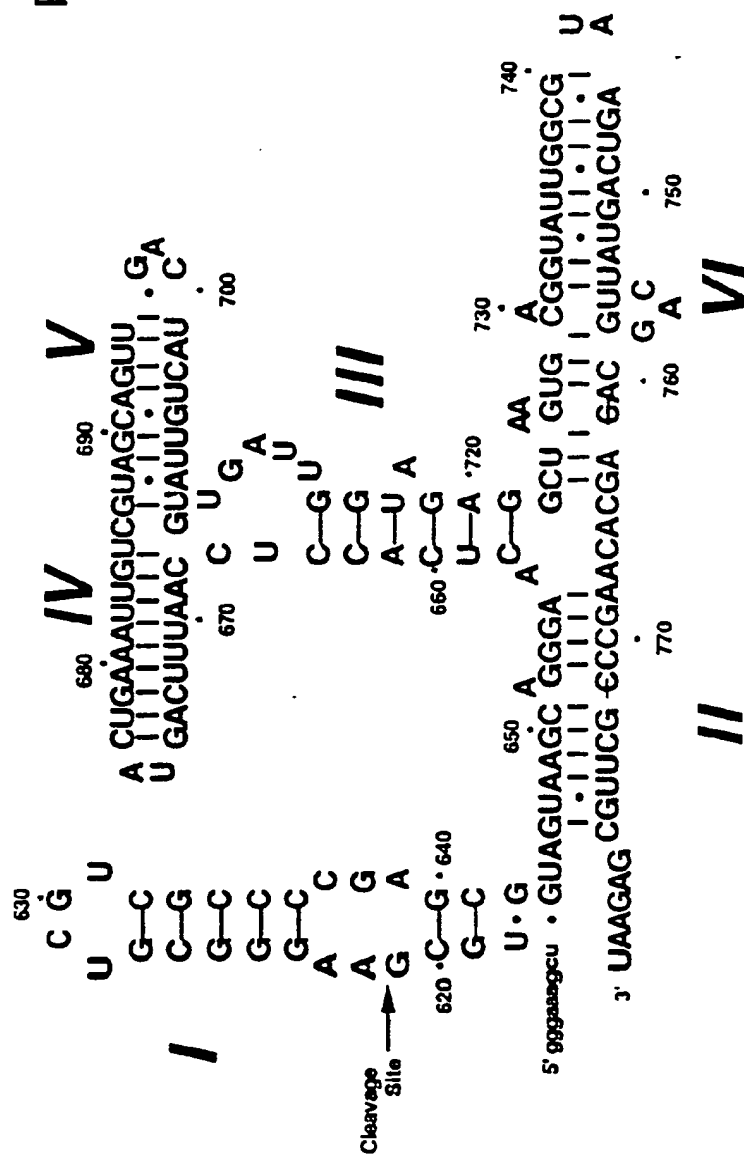


FIG. 4.

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FIG. 5.



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FIG. 6b.

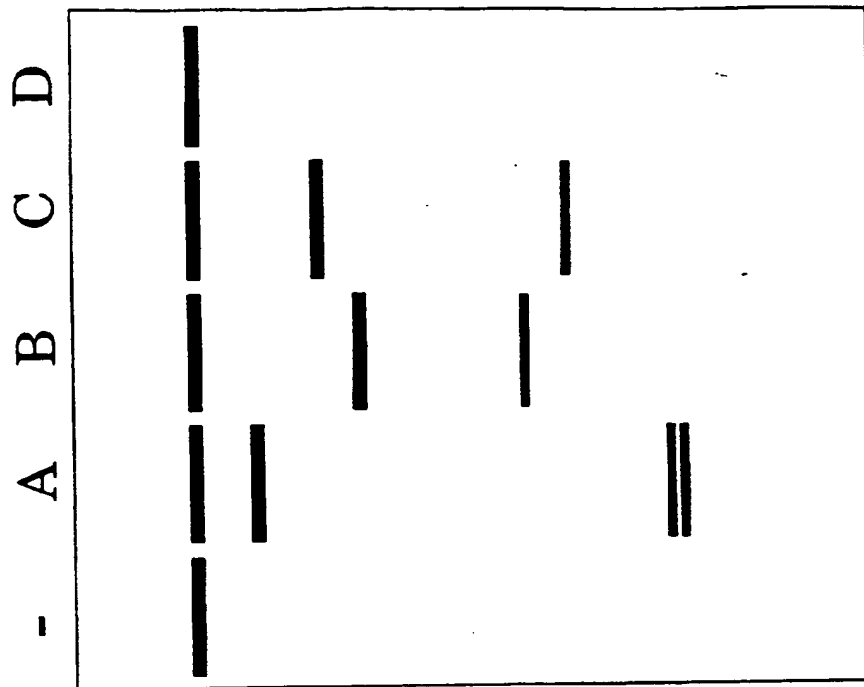
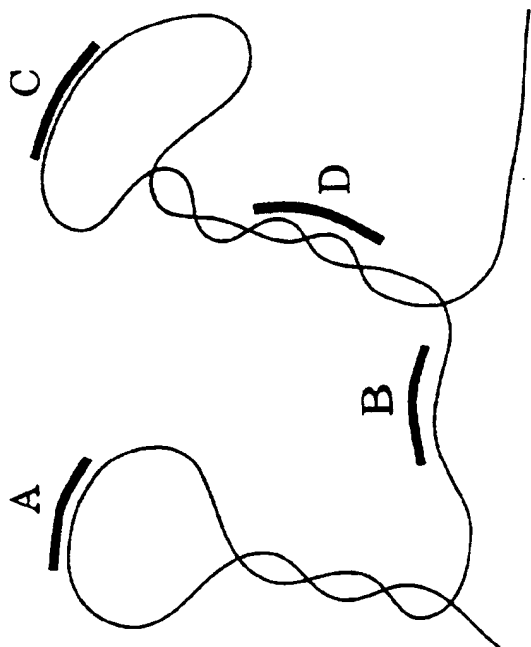


FIG. 6a.



- Body-labeled transcript (not purified)
- DNA oligo (10 nM, 100 nM and 1000 nM)
- RNase H (0.08 - 1.0 u/μl)
- 37°C, 10 min

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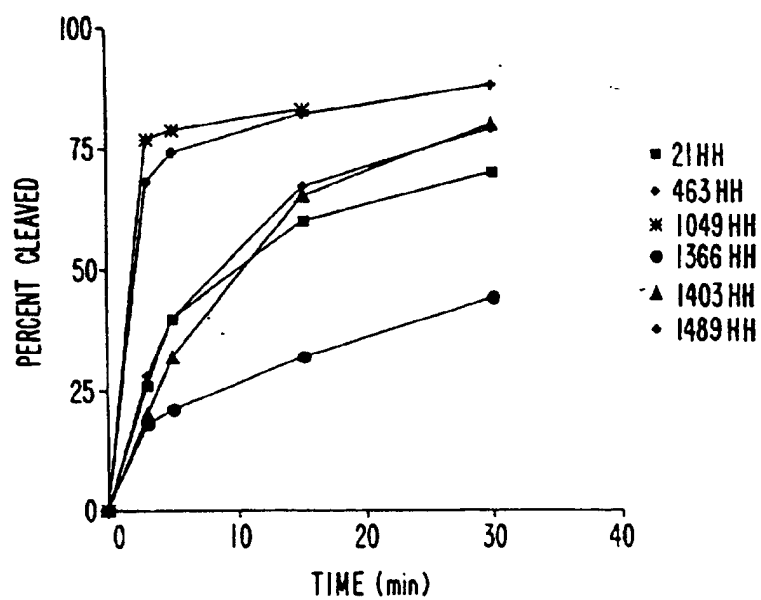


FIG. 7.

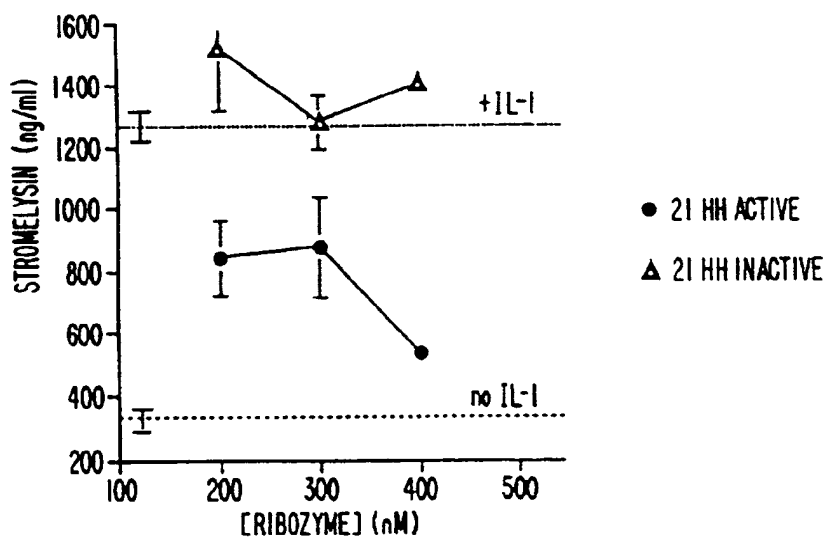


FIG. 8.

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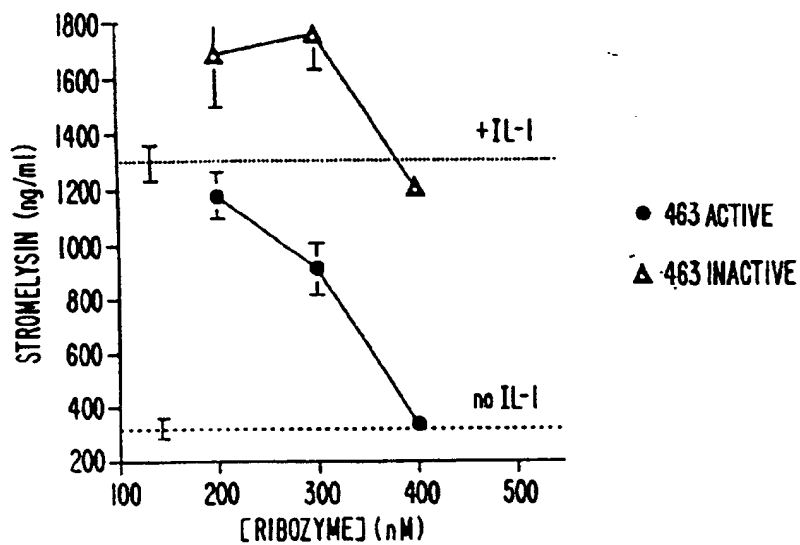


FIG. 9.

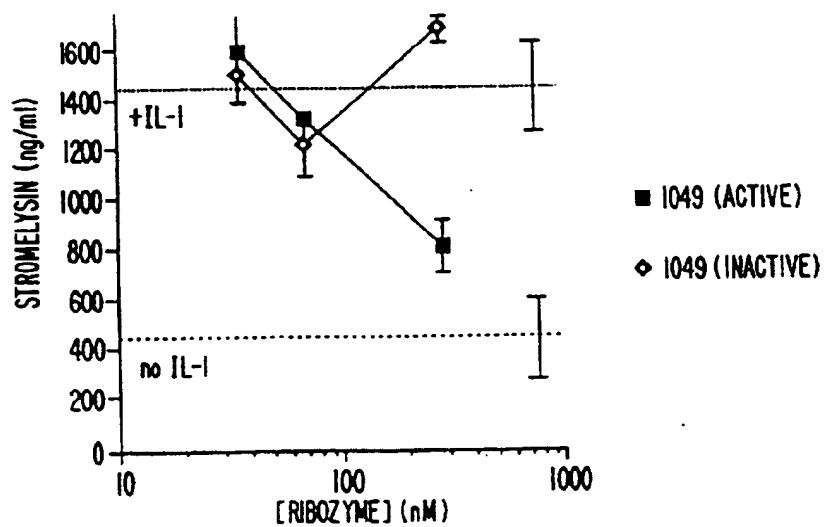


FIG. 10.

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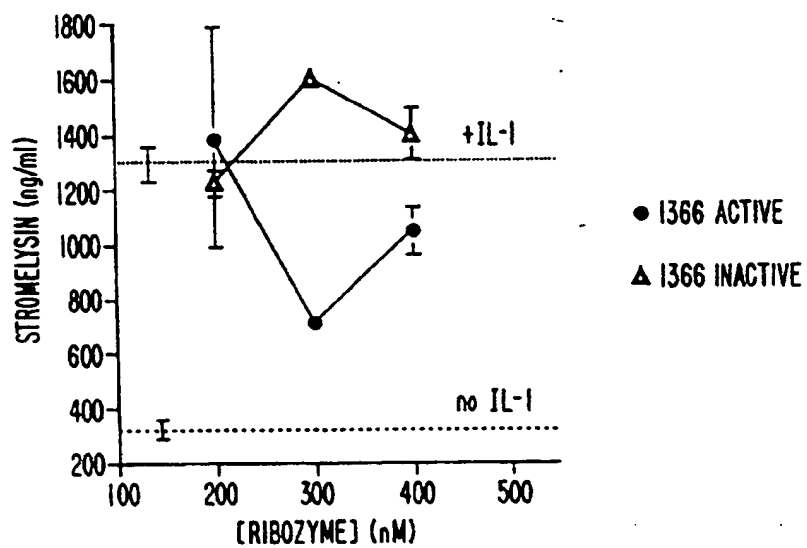


FIG. 11.

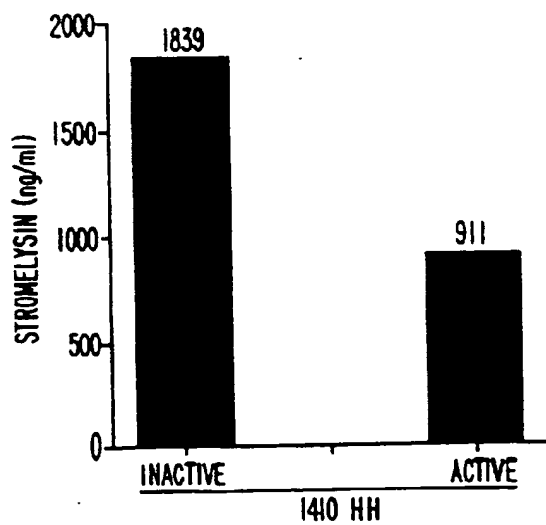


FIG. 12.

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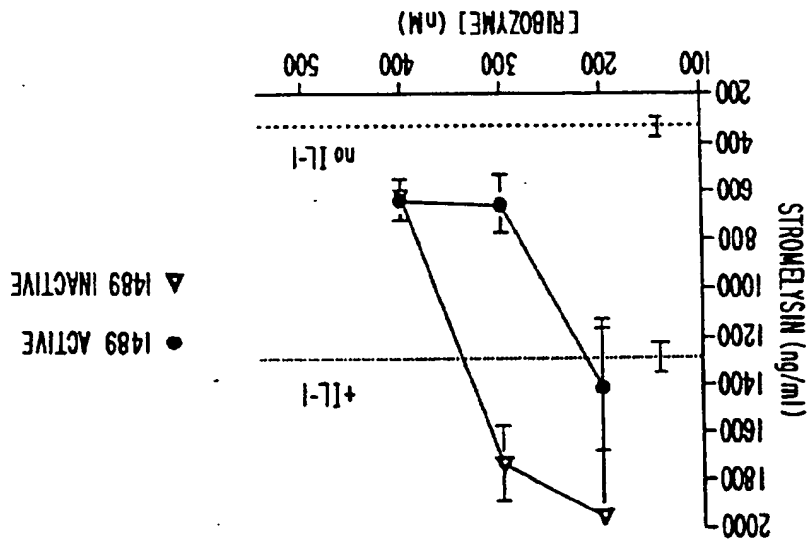


FIG. 13.

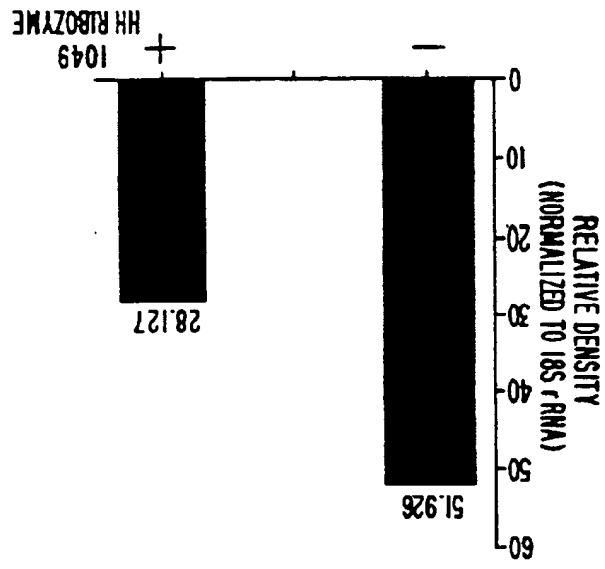


FIG. 14.

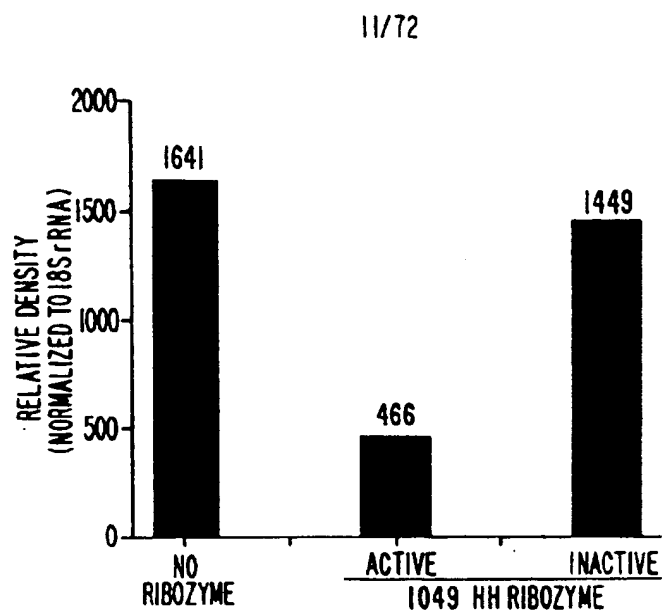


FIG. 15.

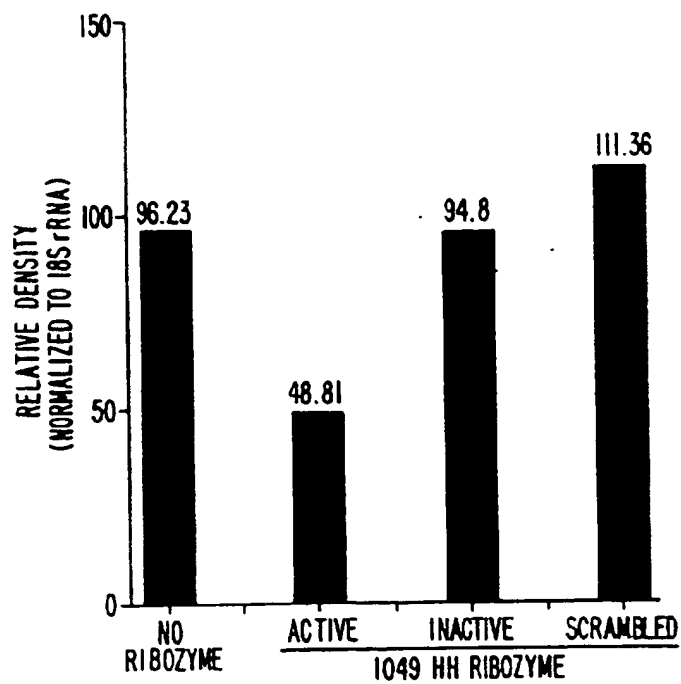
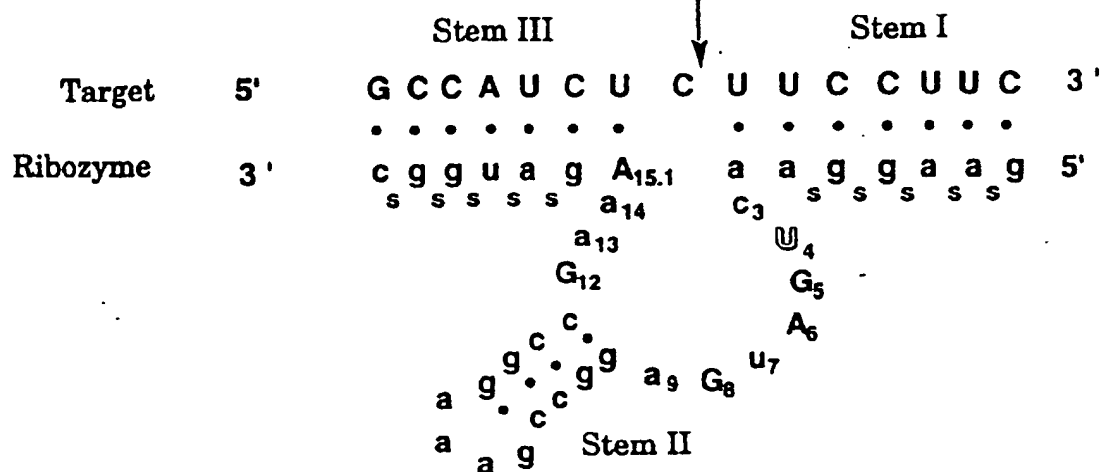


FIG. 16.

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Cleavage Site



Upper case= ribonucleotides

Lower case= 2'-O-methyl nucleotides

U = 2'-C-Allyl modification

s = phosphorothioate linkages

FIG. 17a.

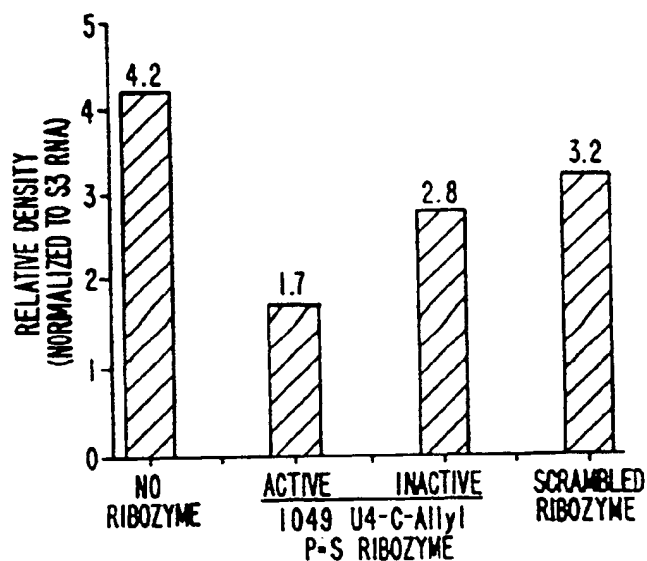


FIG. 17b.

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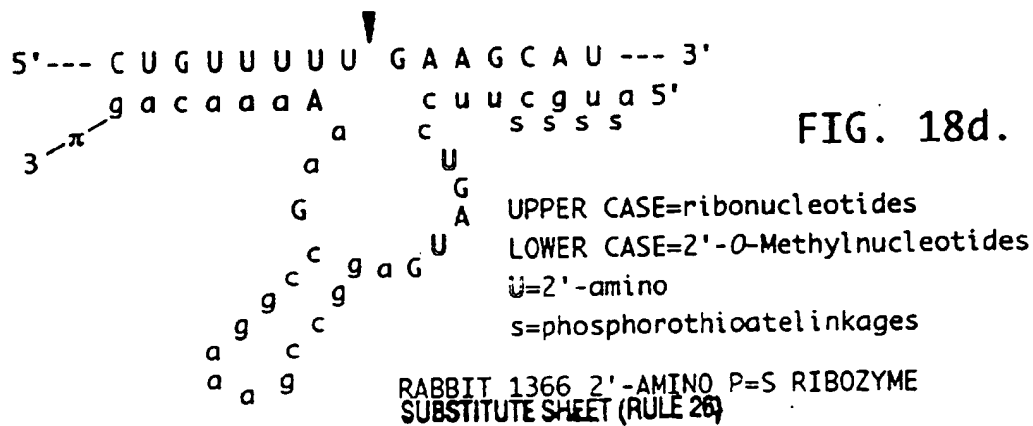
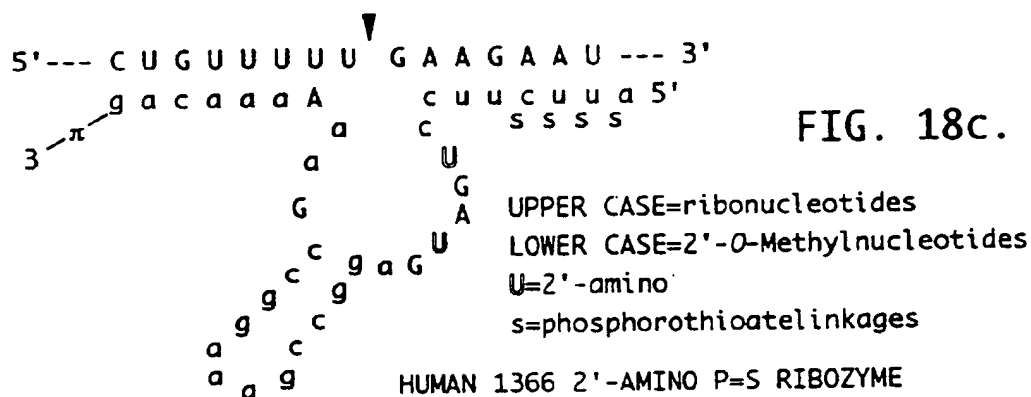
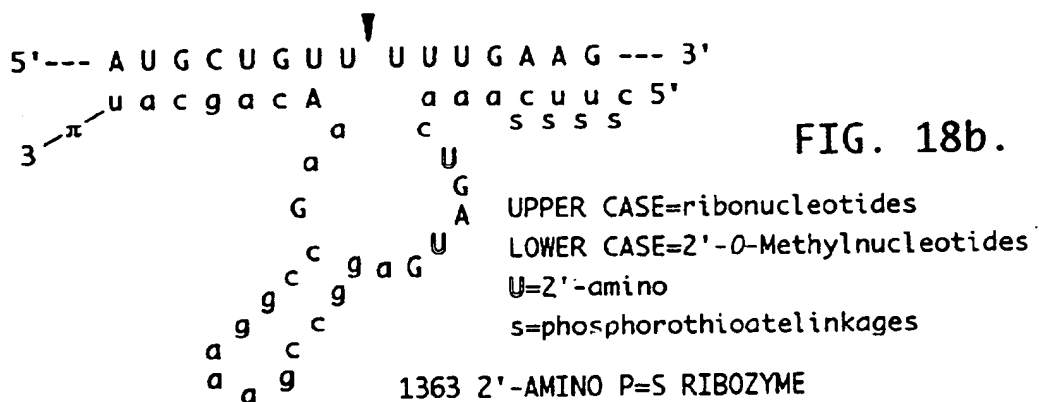
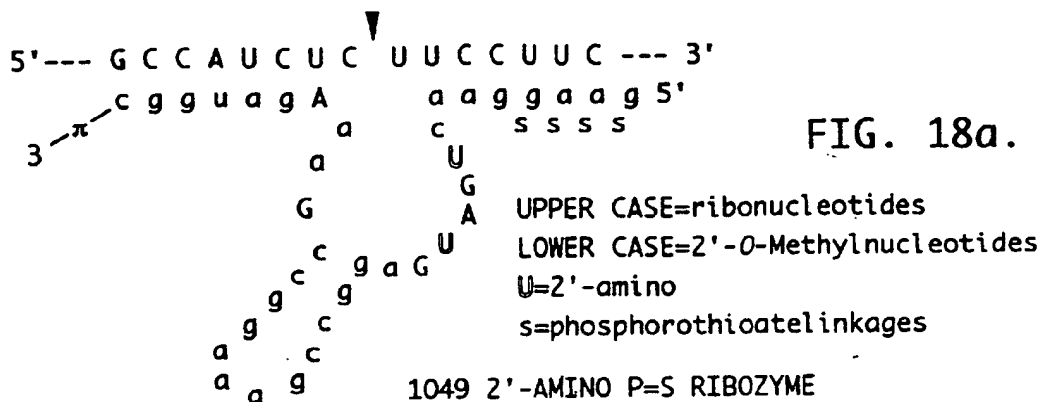


FIG. 19.

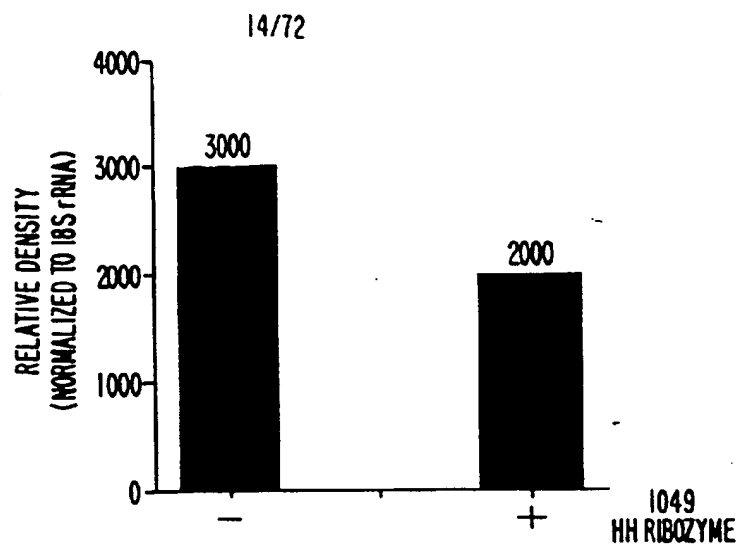


FIG. 20.

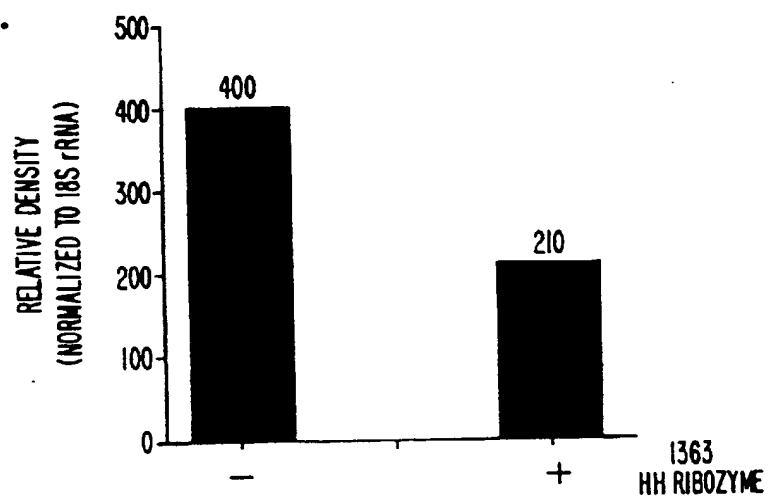
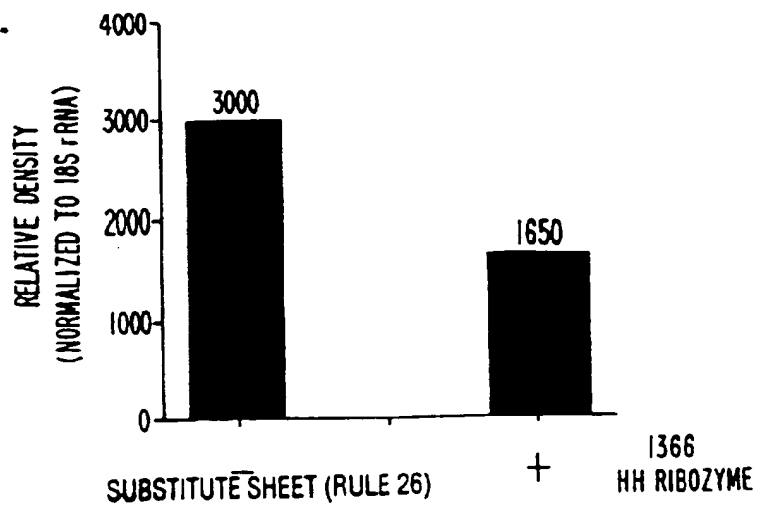
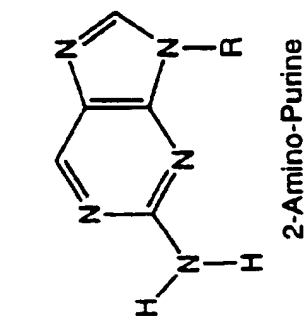


FIG. 21.

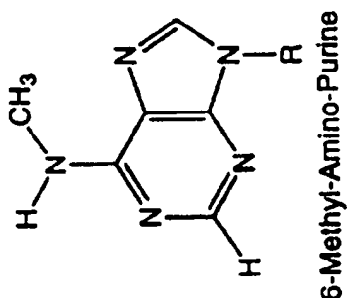


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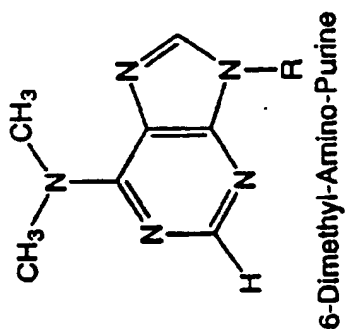
FIG. 22a.



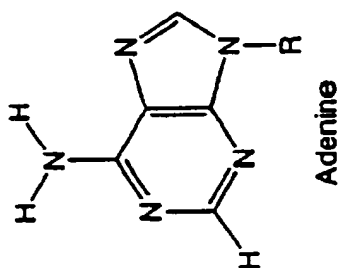
2-Amino-Purine



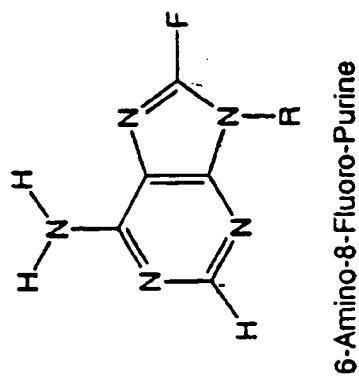
6-Methyl-Amino-Purine



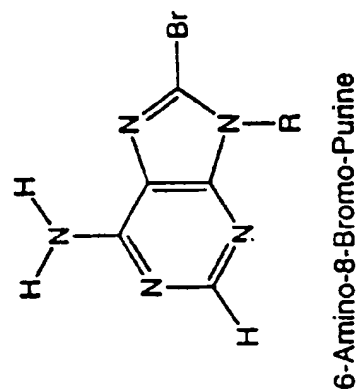
6-Dimethyl-Amino-Purine



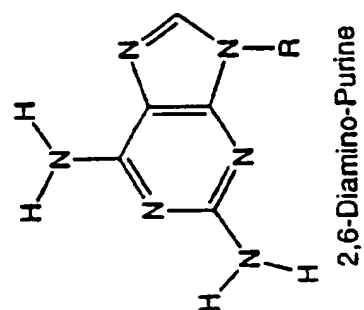
Adenine



6-Amino-8-Fluoro-Purine



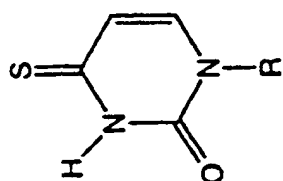
6-Amino-8-Bromo-Purine



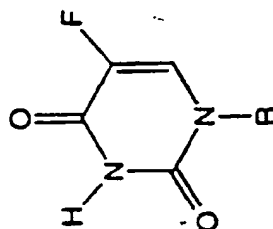
2,6-Diamino-Purine

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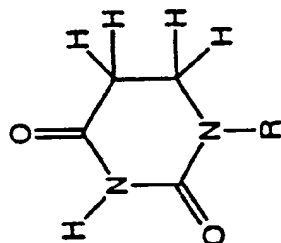
FIG. 22b.



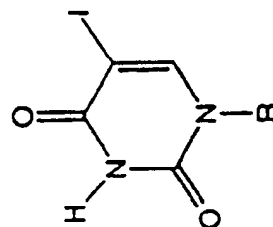
4-Thio-Uracil



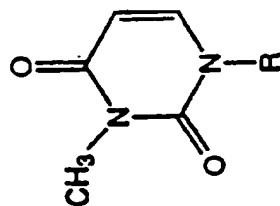
5-Fluoro-Uracil



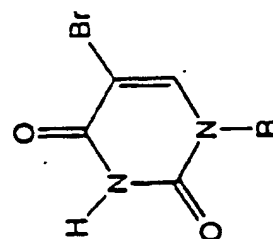
5,6-Dihydro-Uracil



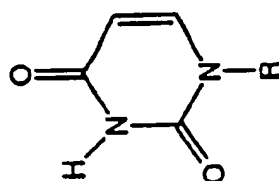
5-Iodo-Uracil



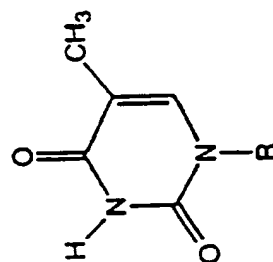
3-Methyl-Uracil



5-Bromo-Uracil



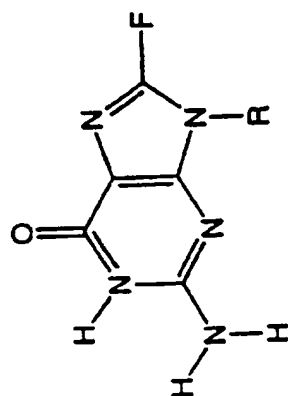
Uracil



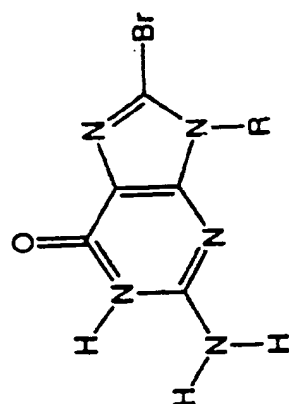
Thymine

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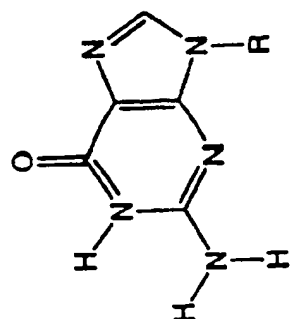
FIG. 22c.



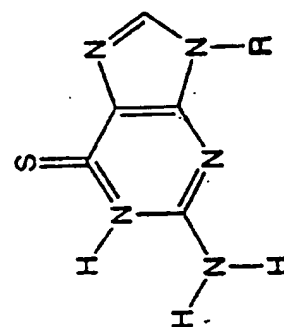
8-Fluoro-Guanine



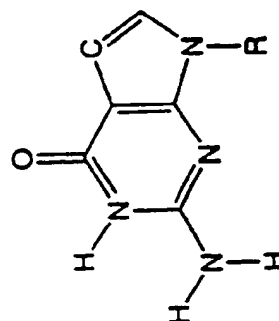
8-Bromo-Guanine



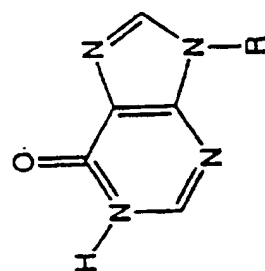
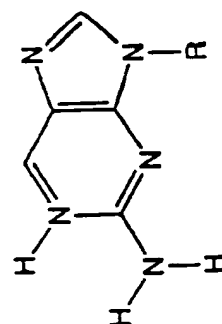
Guanine



6-Thio-Guanine



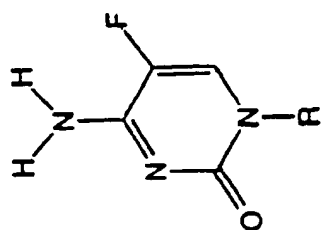
7-Deaza-Guanine

Hypoxanthine
(Inosine base)

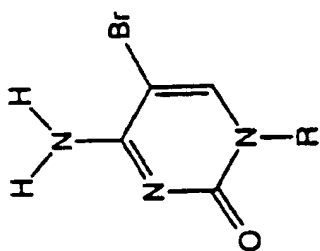
2-Amino-Purine

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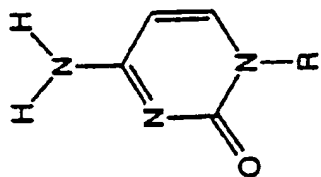
FIG. 22d.



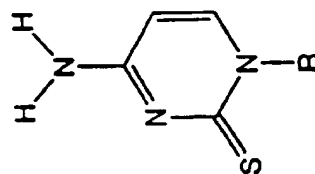
5-Fluoro-Cytosine



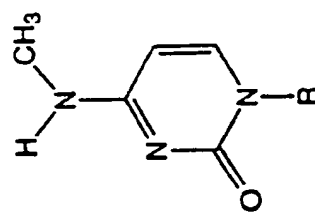
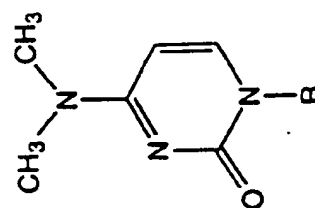
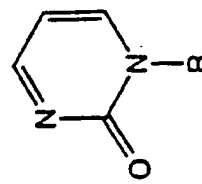
5-Bromo-Cytosine



Cytosine

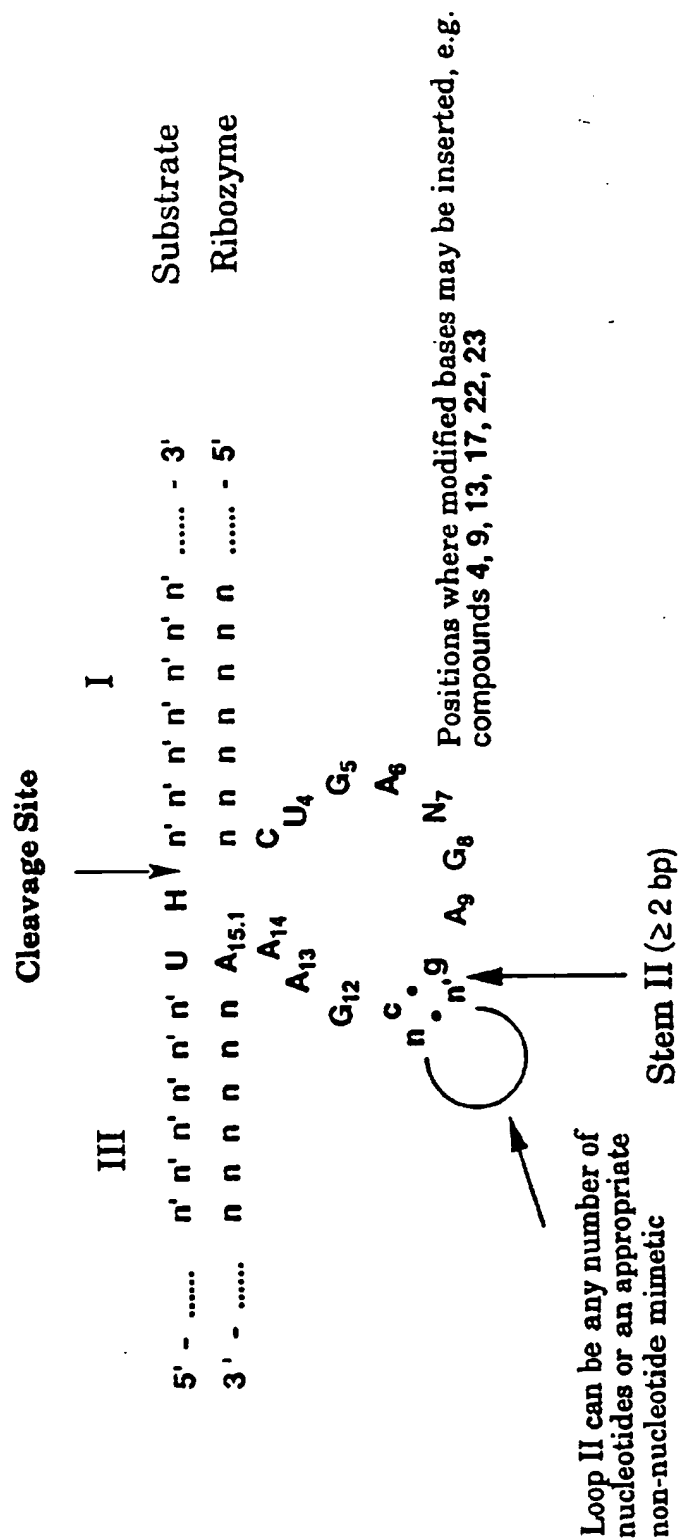


2-Thio-Cytosine

N⁴-Methyl-CytosineN⁴,N⁴-dimethyl-Cytosine

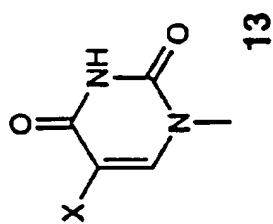
2-Pyridone

FIG. 23.

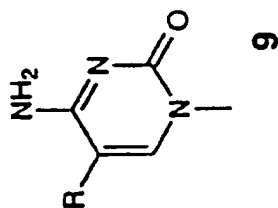


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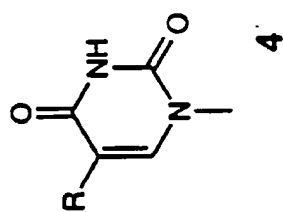
FIG. 24.



5-Halouridine

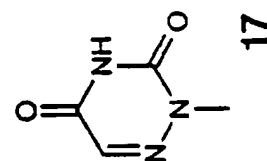
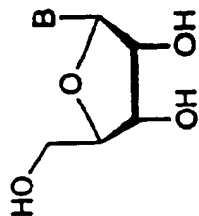


5-Alkylcytidine

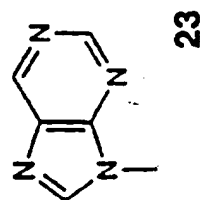


5-Alkyluridine

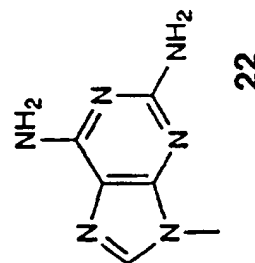
B =



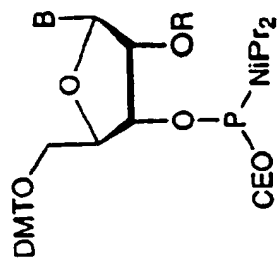
6-Azauridine



Purine

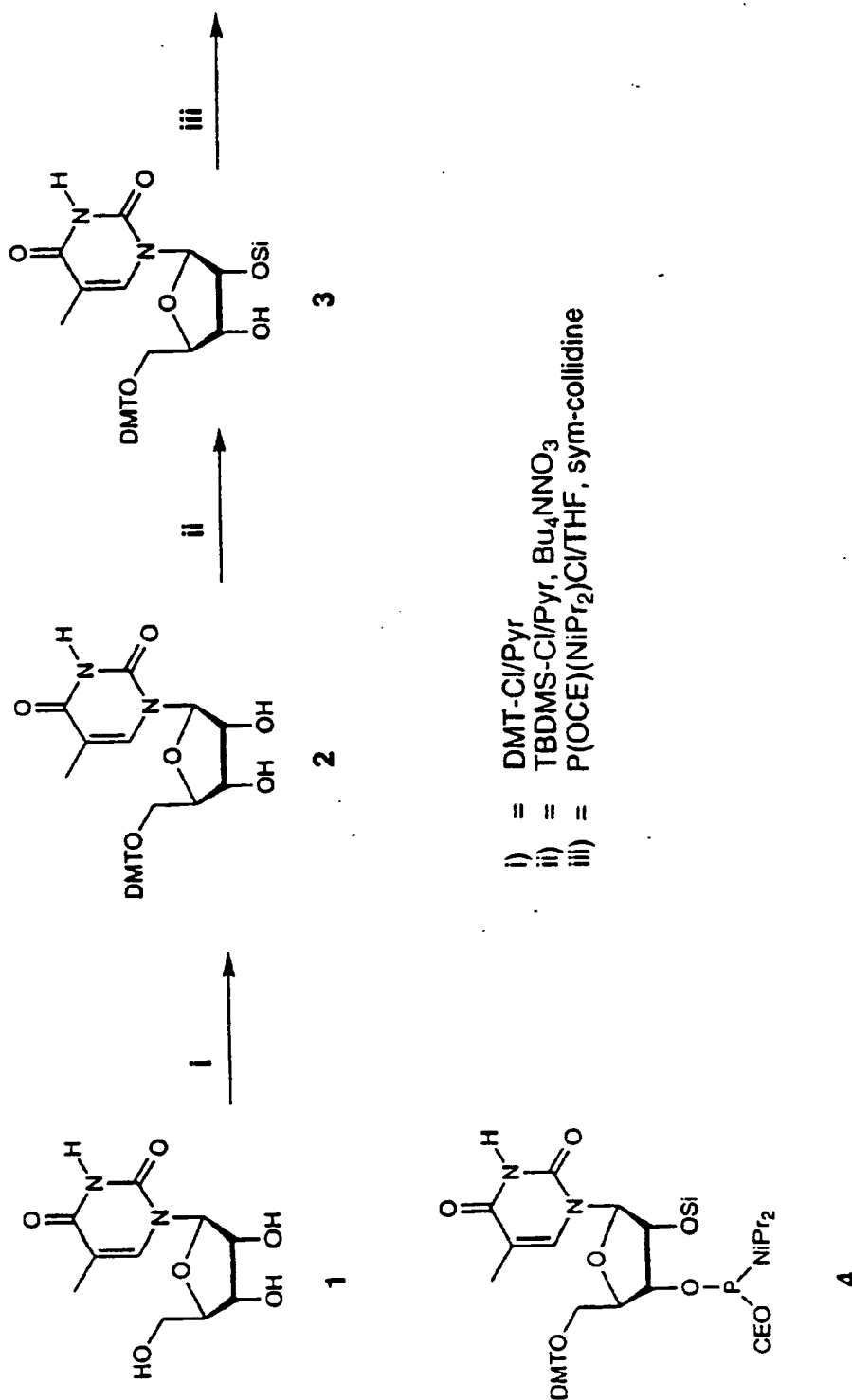


Diaminopurine



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FIG. 25.



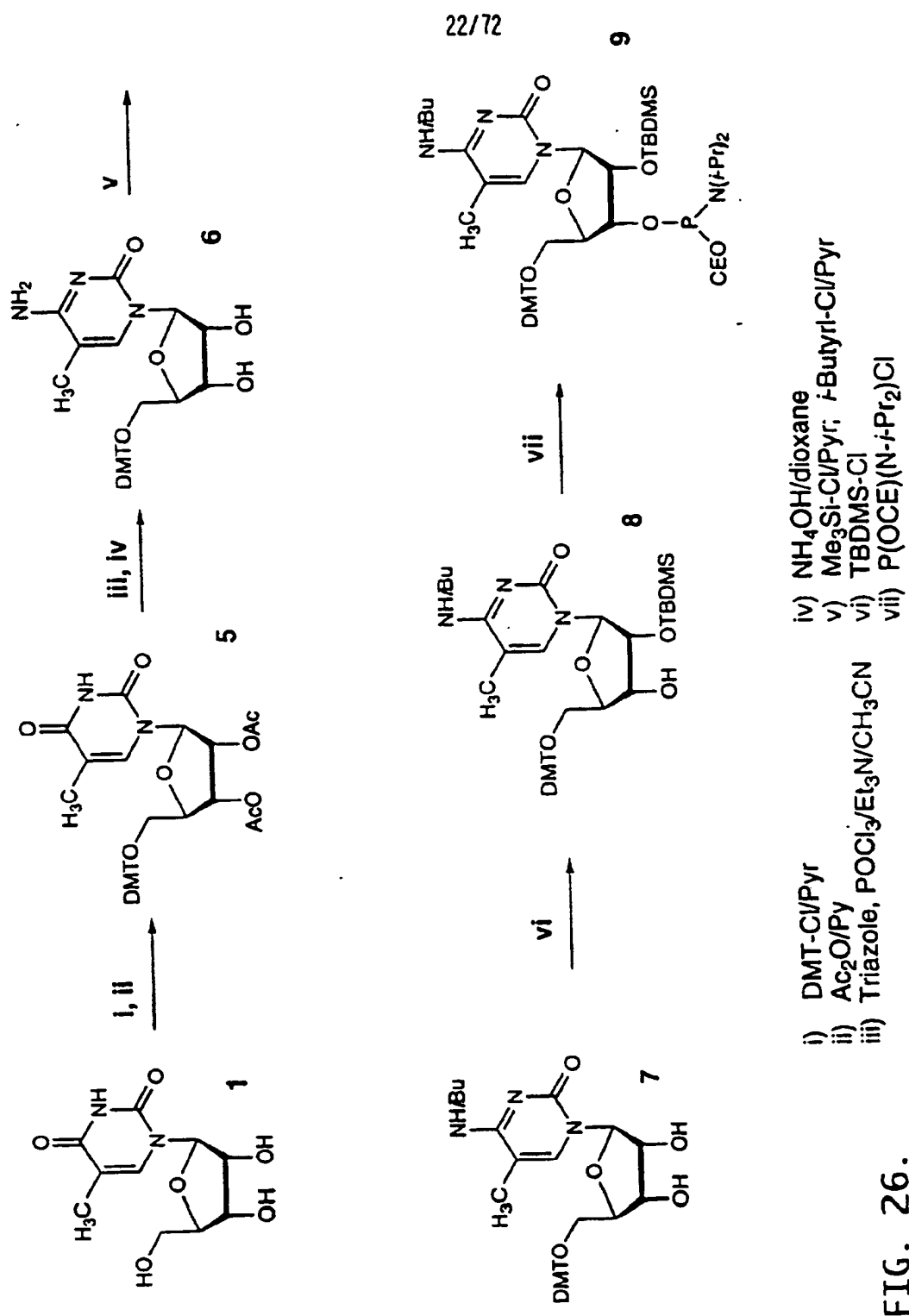
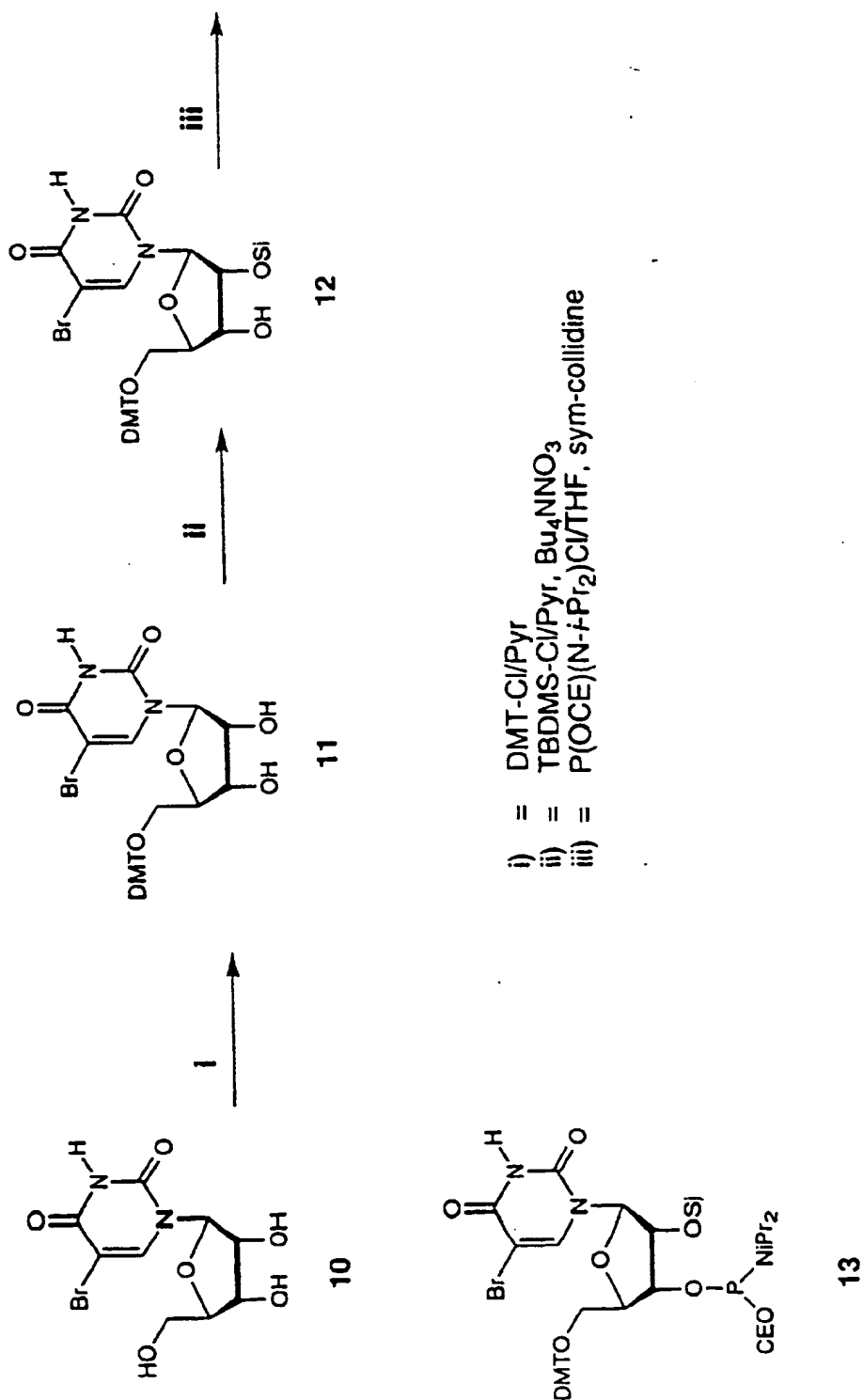


FIG. 26.

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FIG. 27.



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FIG. 28.

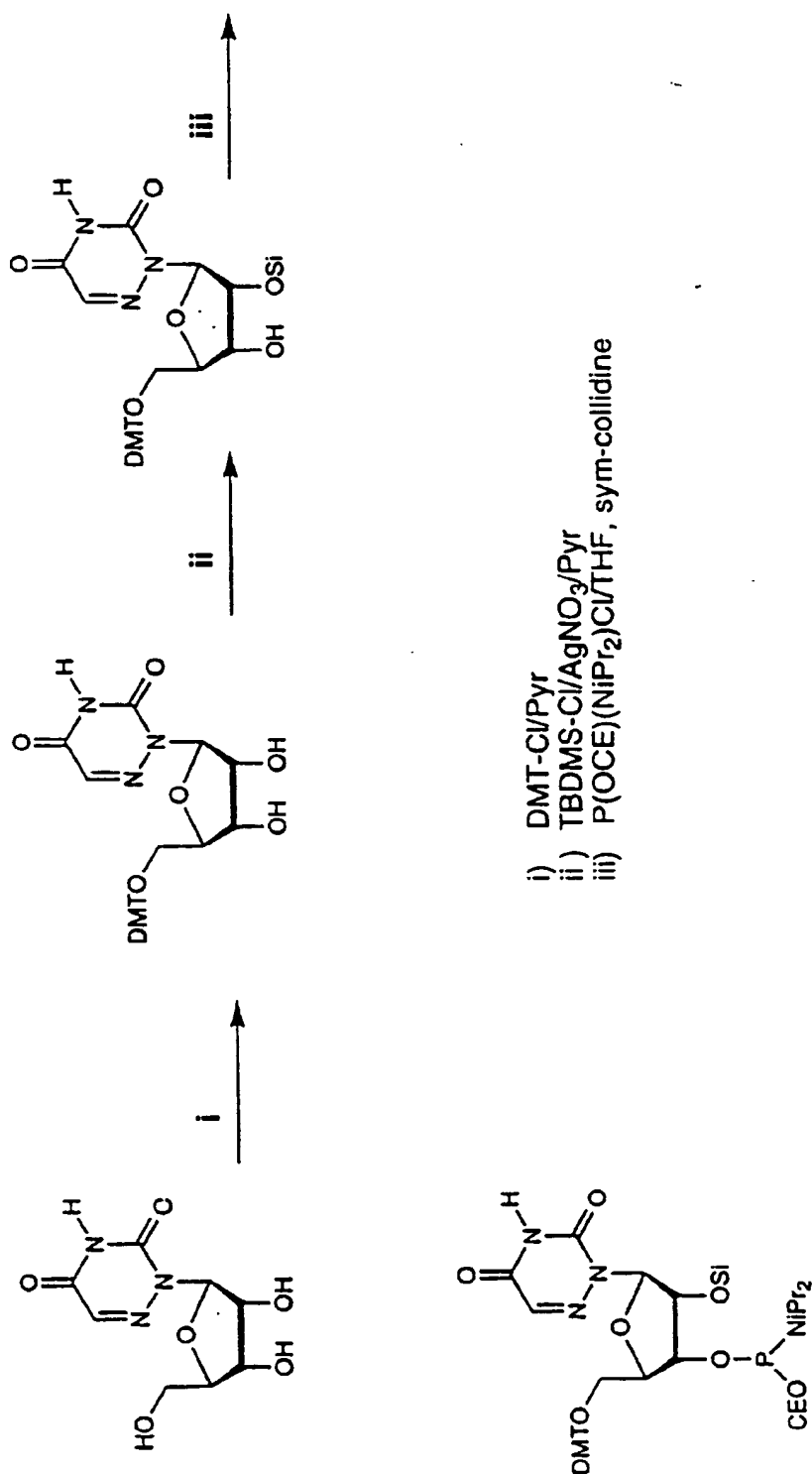
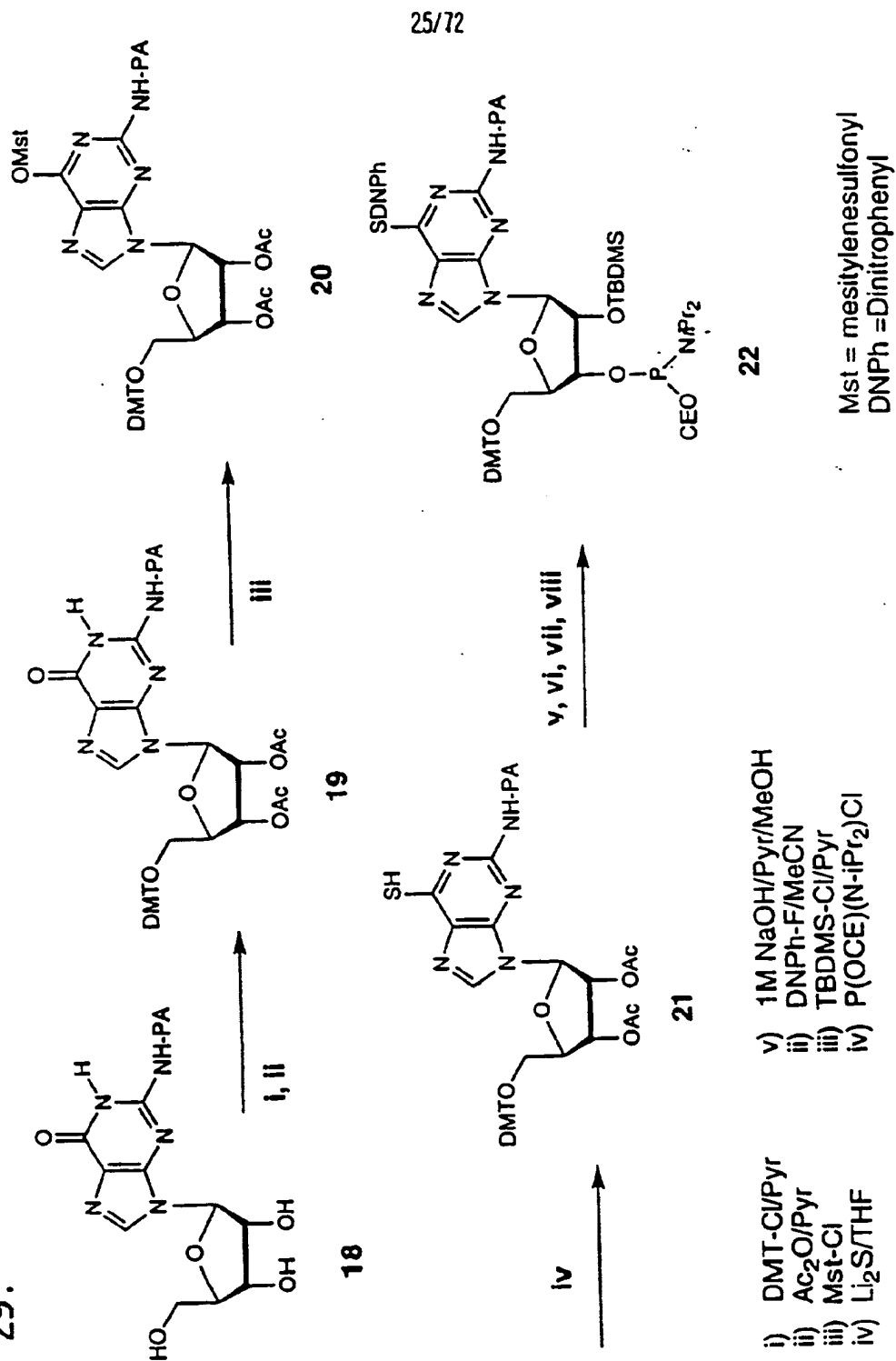
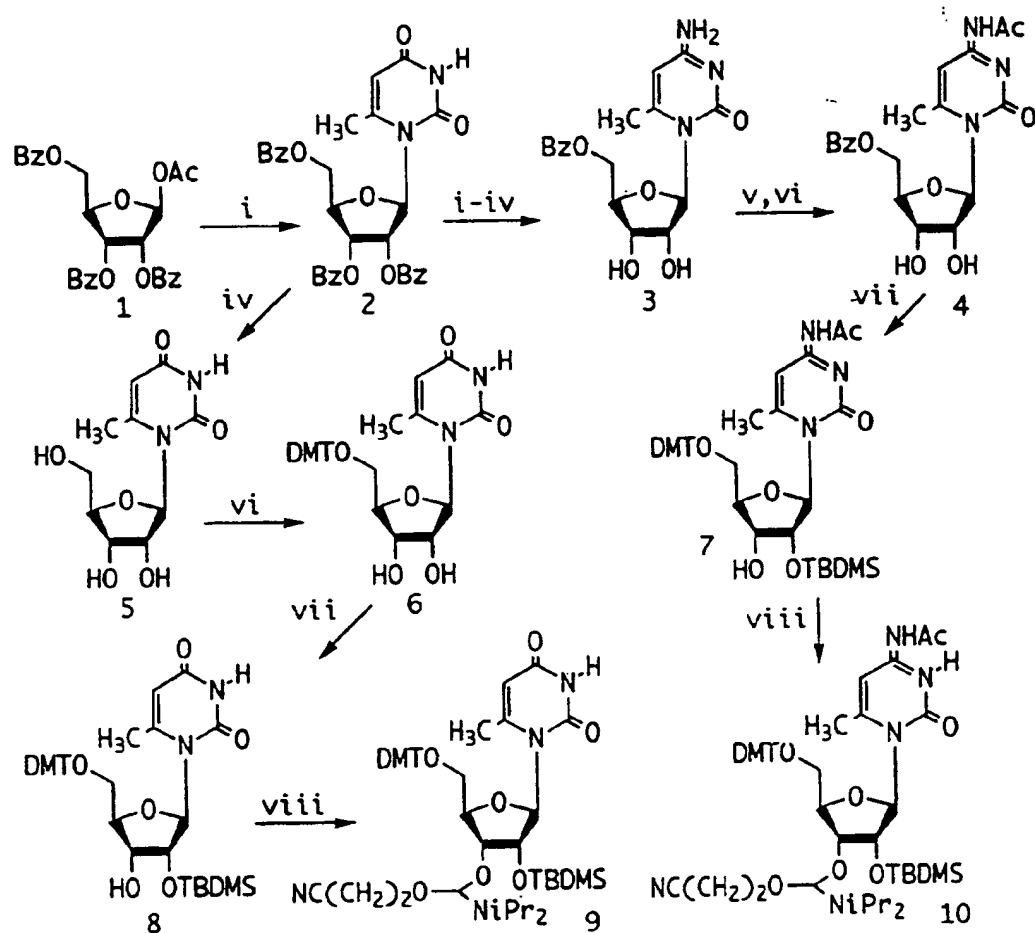


FIG. 29.



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REAGENTS AND CONDITIONS:

- i) 6-Me-Ura^{TMS}, CF₃SO₃SIME₃, 0°C;
- ii) 1,2,4-triazole, POCl₃; iii) NH₄OH/dioxane;
- iv) 2M NaOH/Pyr/MeOH; v) MeSI-CL/PYR, THEN AC₂O;
- vi) DMT-Cl/Pyr;
- vii) TBDMS-Cl/AgNO₃/Pyr/THF;
- viii) 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite, DIPEA/CH₂Cl₂.

FIG. 30.

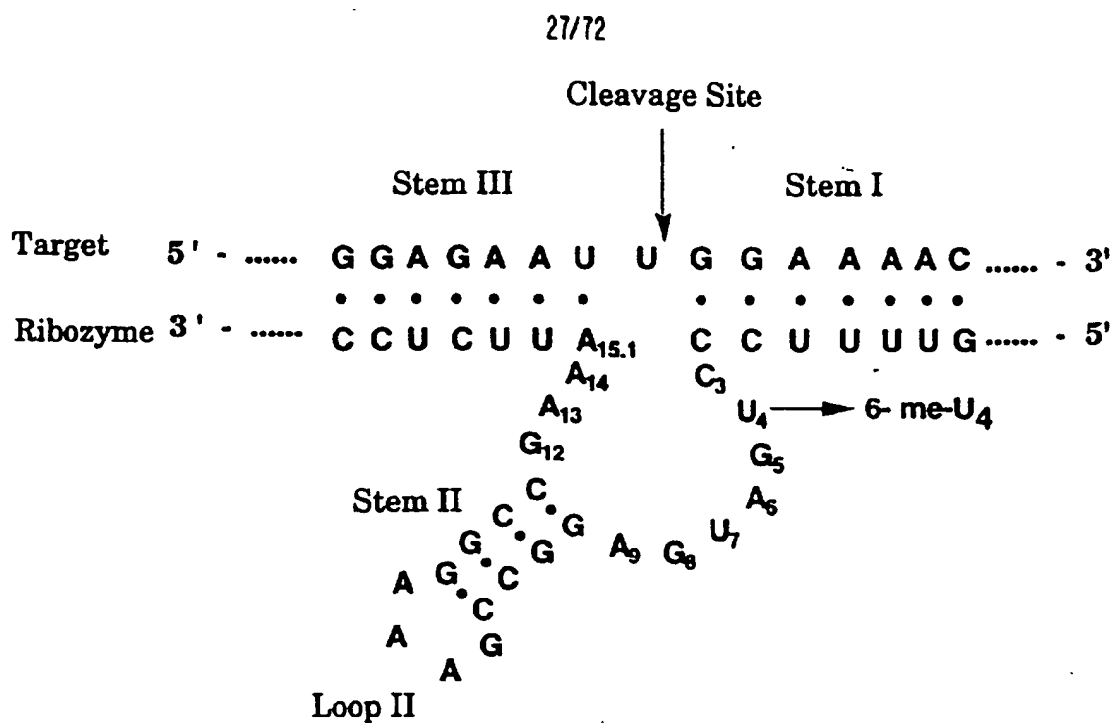
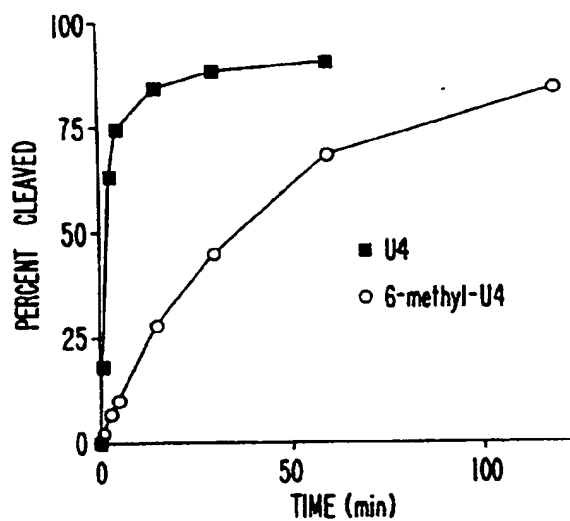


FIG. 31.



[Ribozyme]=40nM [Substrate]=~1nM

FIG. 32.

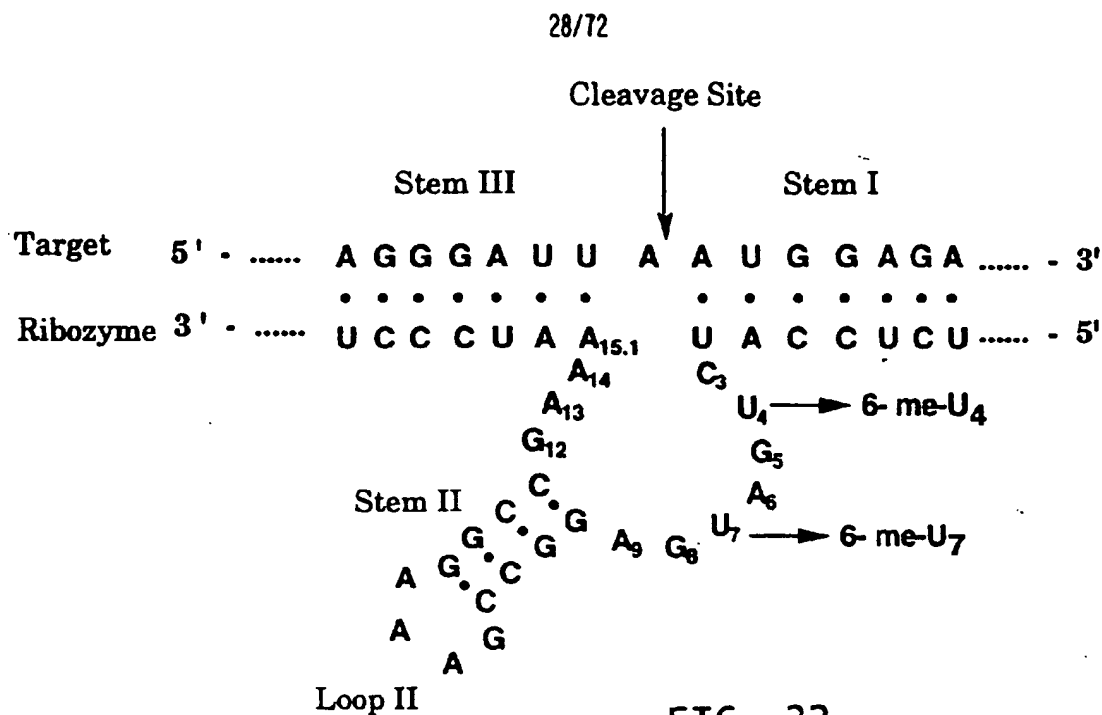
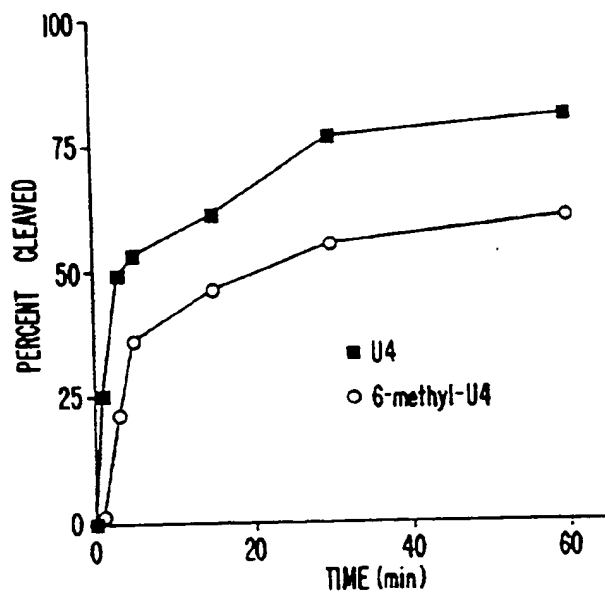


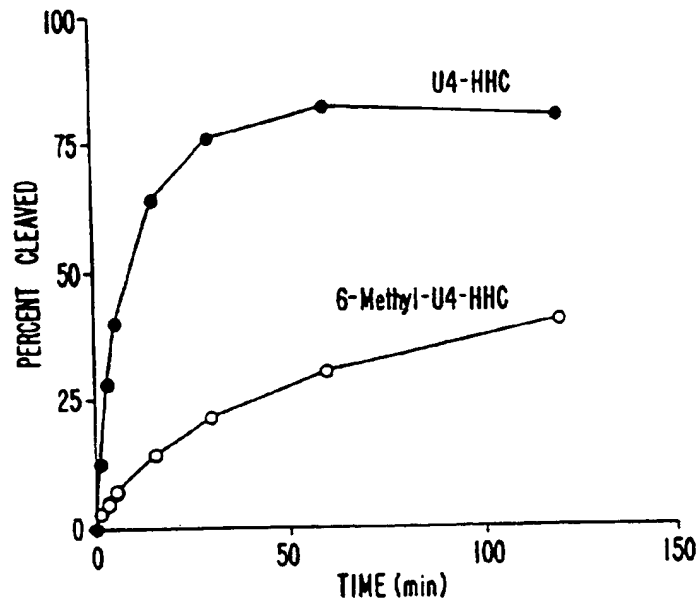
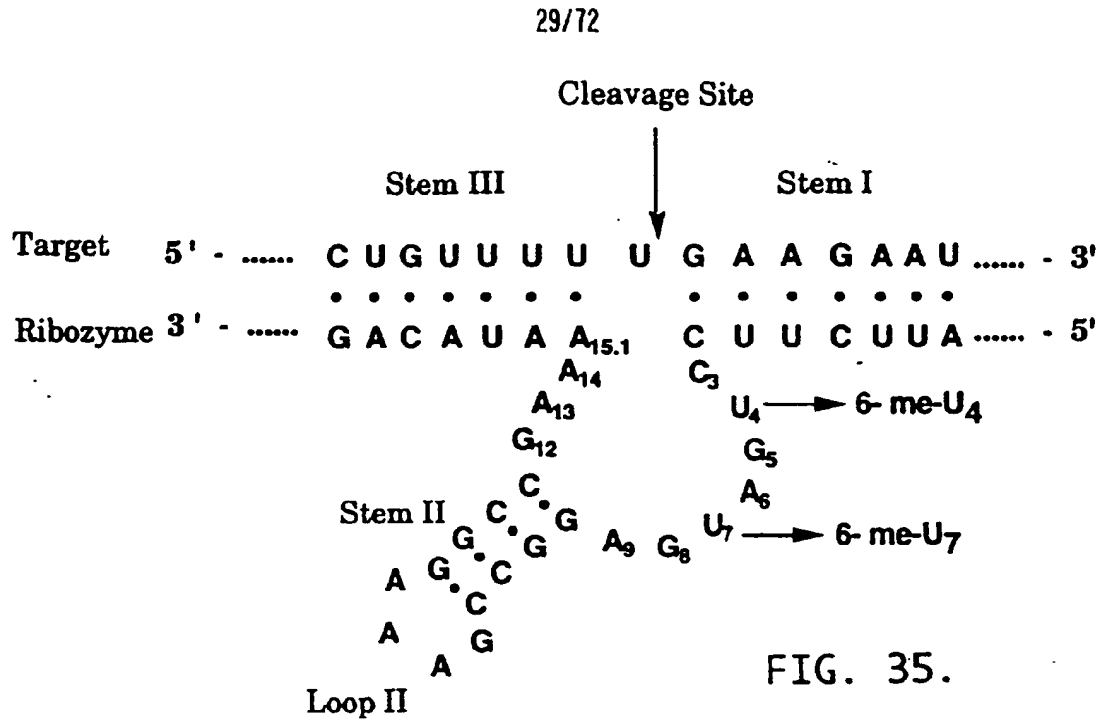
FIG. 33.



[Ribozyme]=40nM [Substrate]=~1nM

FIG. 34.

SUBSTITUTE SHEET (RULE 26)



[Ribozyme]=40nM [Substrate]=~1nM

FIG. 36.
SUBSTITUTE SHEET (RULE 26)

FIG. 37.

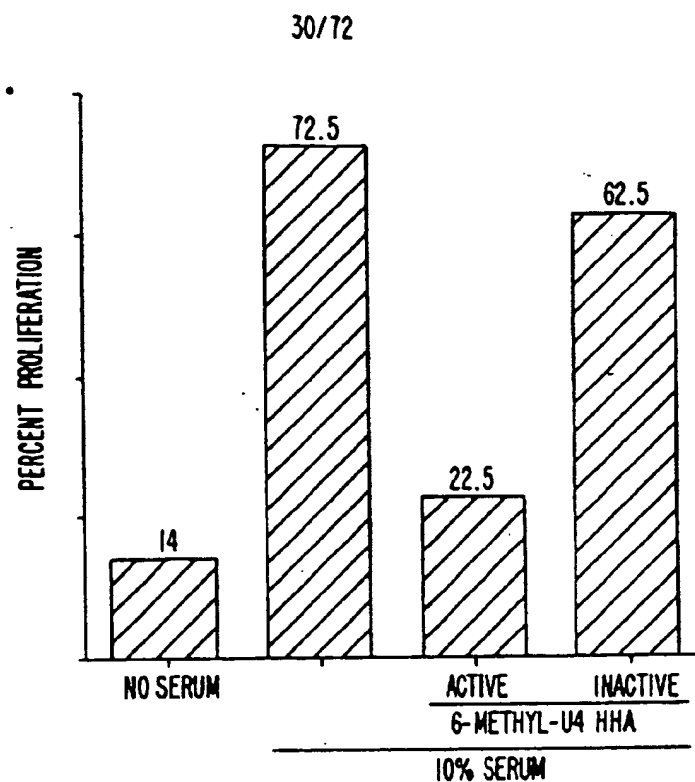
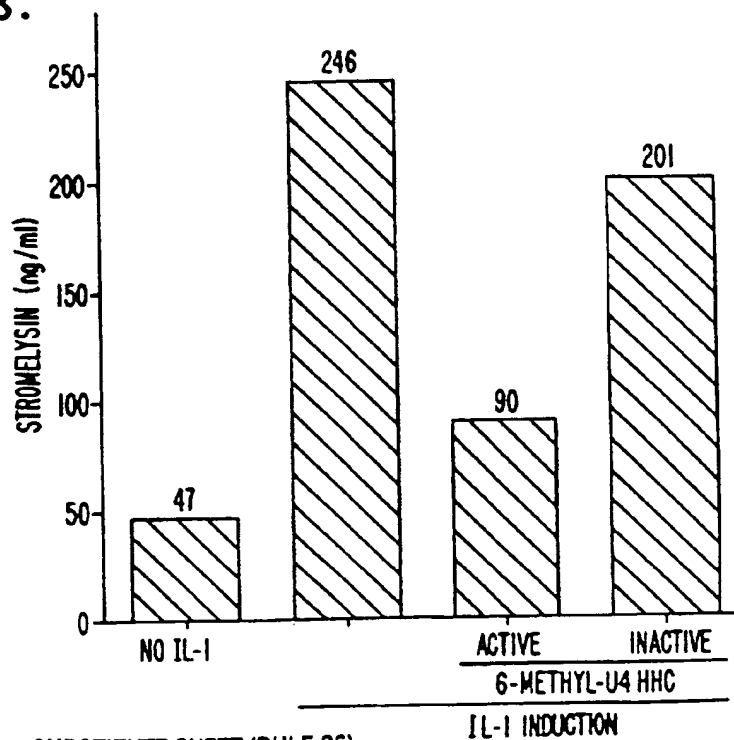
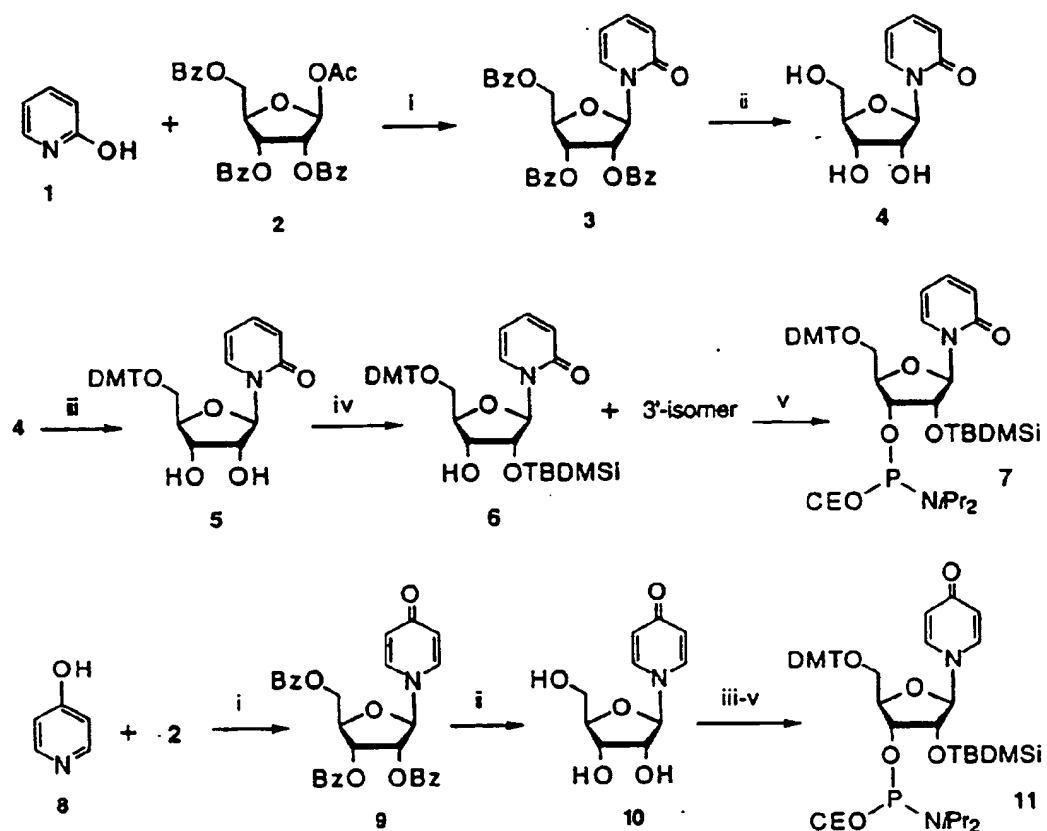


FIG. 38.



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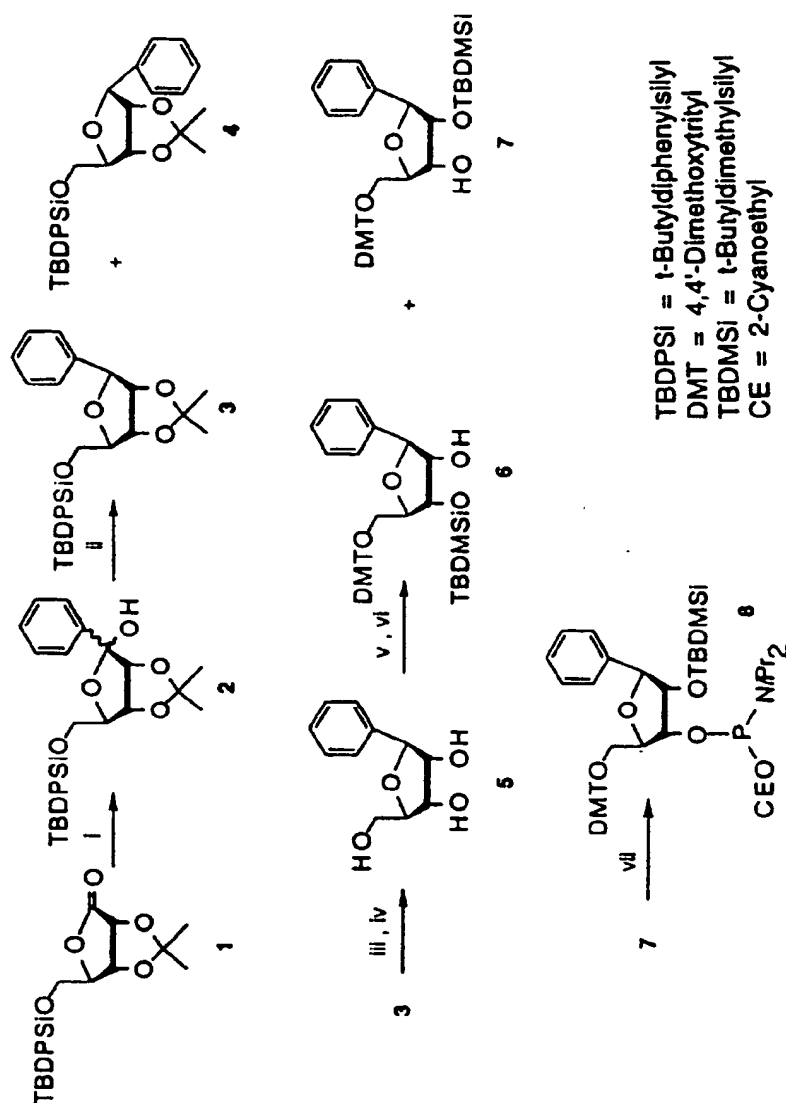


Bz = Benzoyl
 Ac = Acetyl
 DMT = 4,4'-Dimethoxytrityl
 TBDMSi = *t*-Butyldimethylsilyl
 CE = 2-cyanoethyl

Reagents and Conditions: i: N,O-bis(trimethylsilyl)acetamide (BSA)/TMSTf/
 CH₃CN, 70 °C, ii: NaOCH₃/CH₃OH, iii: DMT-Cl/DMAP/Et₃N/Pyr, iv: TBDMSi-
 Cl/AgNO₃/Pyr/THF, v: P(OCE)(N-*i*Pr₂)Cl/DIPEA/1-MeIm/CH₂Cl₂.

FIG. 39.

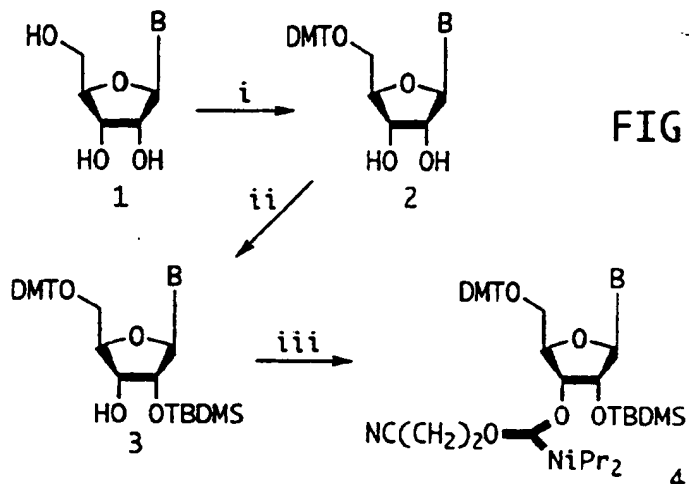
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Reagents and Conditions: i: PhLi/THF, -78 °C, ii: Et₃SiH/BF₃·Et₂O/CH₃CN, -40 °C,
 iii: 1M TBAF/THF, iv: 70 % aq. CH₃COOH, v: DMT-C/DMAPE/Et₃N/Pyr, vi:
 TBDMSi-C/AgNO₃/Pyr/THF, vii: P(OCE)(N-iPr₂)C/DIPEA/1-MeIm/CH₂Cl₂.

FIG. 40.

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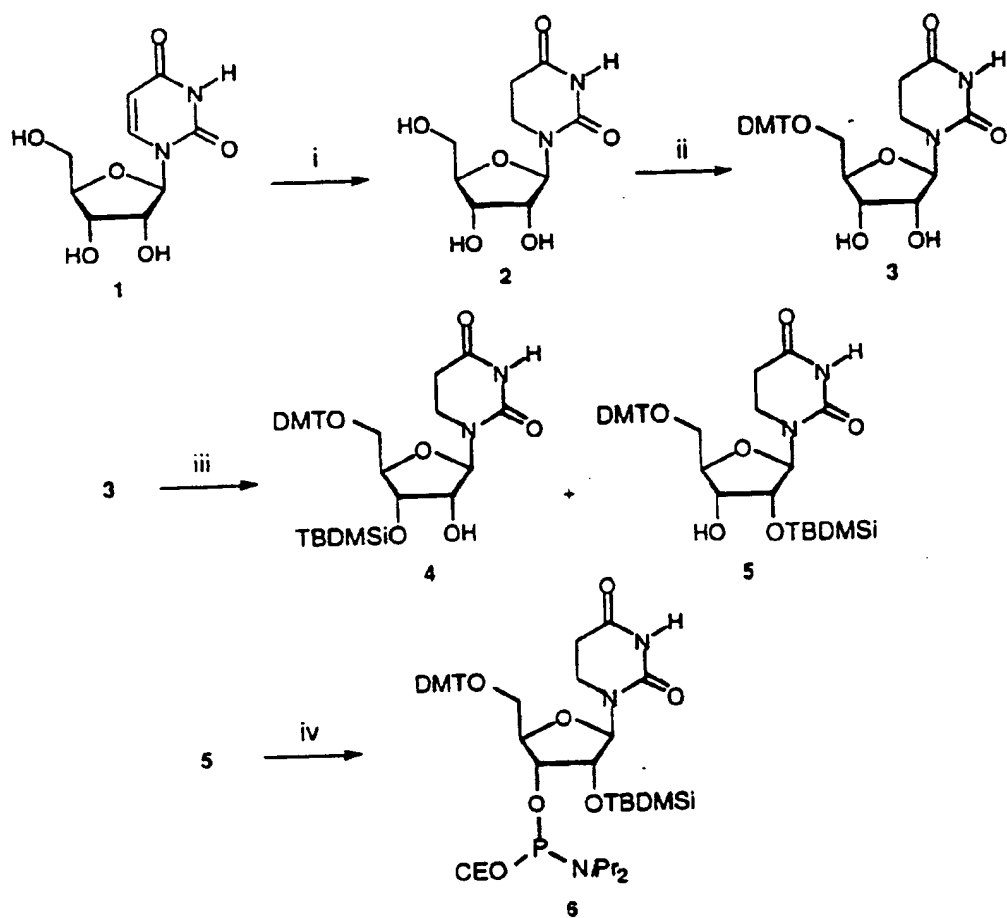
B=Pseudo U,2,4,6-trimethoxy benzene or 3-methyl U

REAGENTS AND CONDITIONS:

- i) DMT-Cl/Pyr;
- ii) TBDMS-Cl/AgNO₃/Pyr/THF;
- iii) 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite, DIPEA/CH₂Cl₂.

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FIG. 42.



DMT = 4,4'-Dimethoxytrityl
 TBDMSi = t-Butyldimethylsilyl
 CE = 2-Cyanoethyl

Reagents and Conditions: i: Pd/Rh, H₂ 60 psi, ii: DMT-Cl/DMAP/Et₃N/Pyr,
 iii: TBDMSi-Cl/AgNO₃/Pyr/THF, iv: P(OCE)(N-iPr₂)Cl/DIPEA/1-MeIm/CH₂Cl₂

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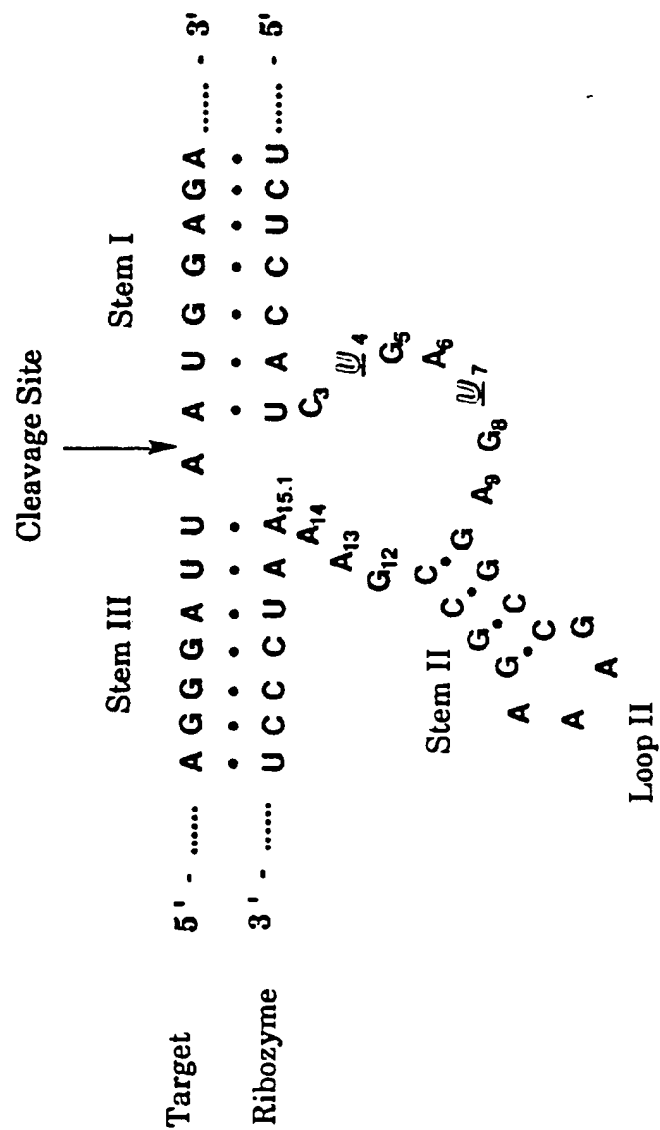
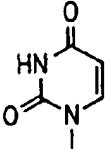
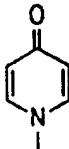
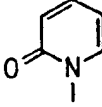

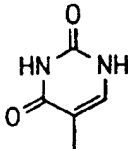
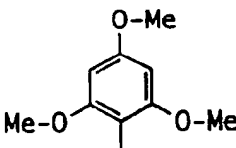
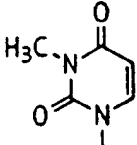


FIG. 43a.

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FIG. 43b.

<u>BASE MODIFICATIONS</u>		<u>HH nt. POSITION</u>	
		<u>4</u>	<u>7</u>
		<u>$k_{obs}(\text{min}^{-1})$</u>	
	U	2.1	2.1
	Pyridin-4-one	0.04	≥ 10
	Pyridin-2-one	0.03	1.2
	Phenyl	0.05	2.5
	Pseudo U	1.0	0.22
	3-O-Methoxy Benzene	0.02	0.14
	3-Me thyl U	0.02	4.6

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FIG. 44a.

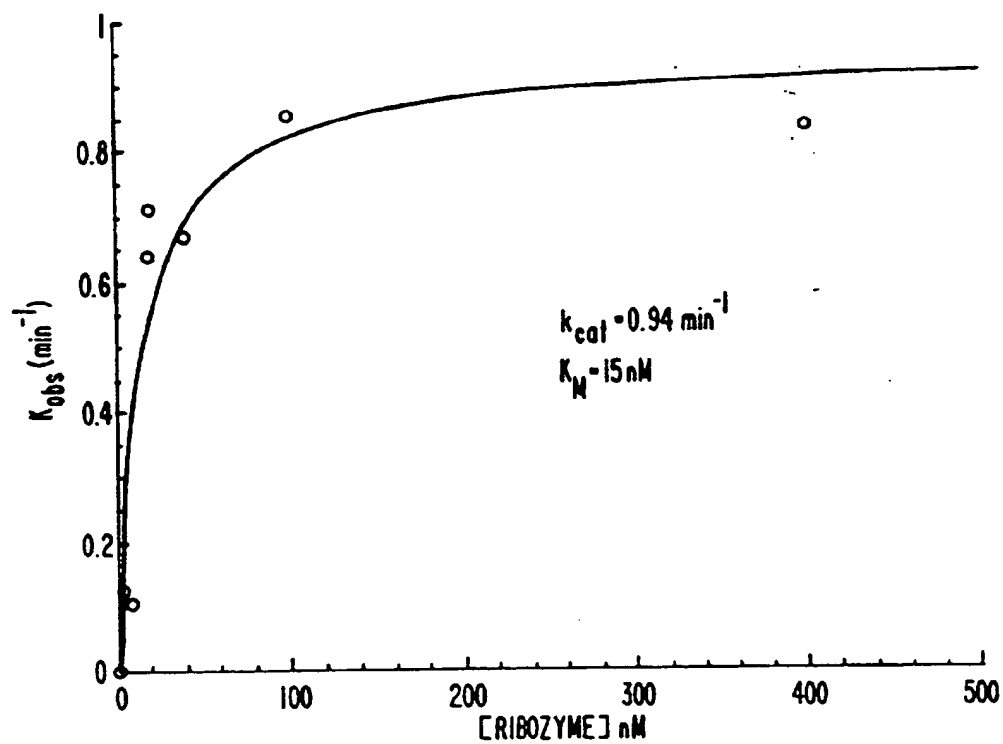
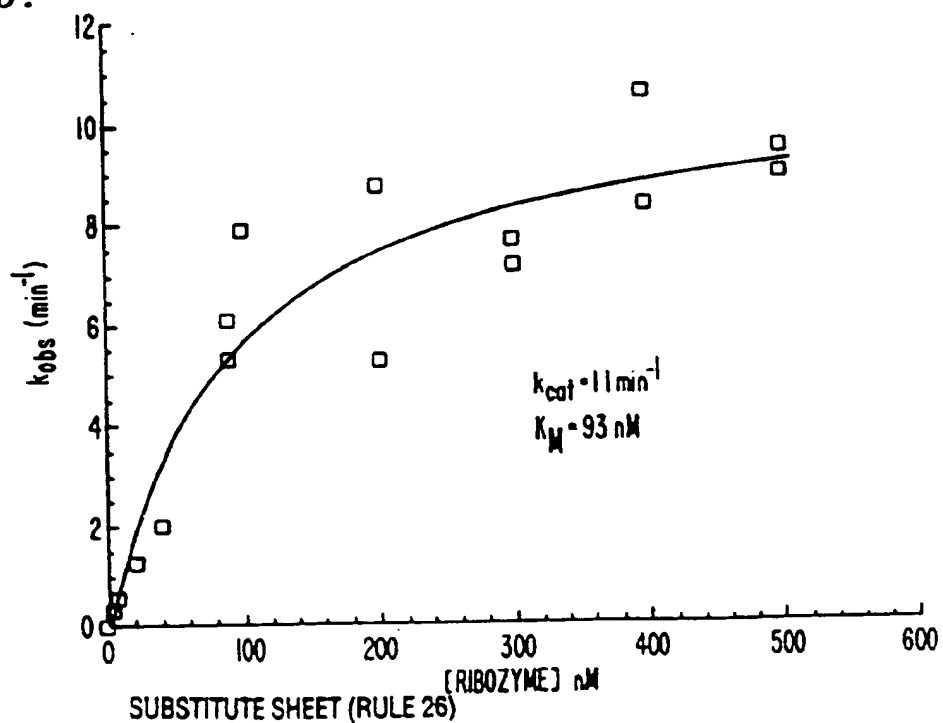


FIG. 44b.



SUBSTITUTE SHEET (RULE 26)

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FIG. 44c.

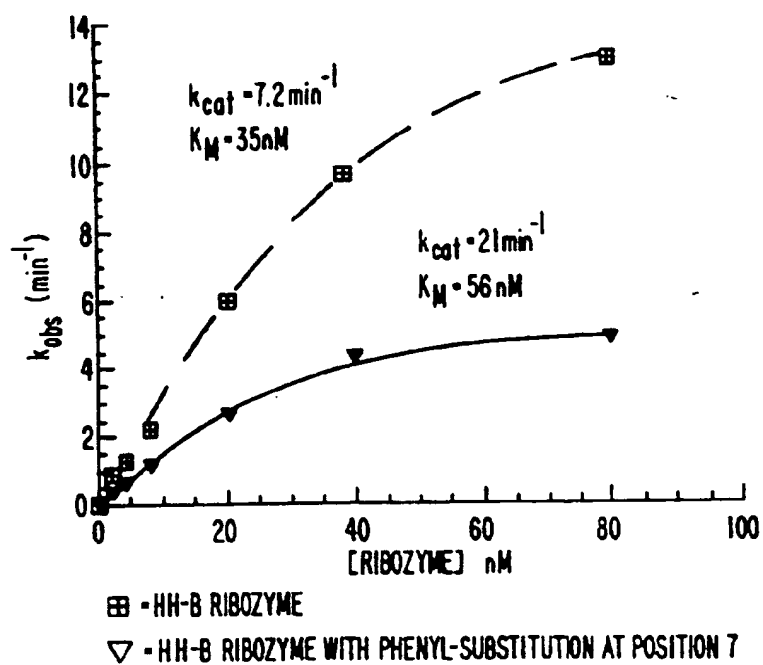
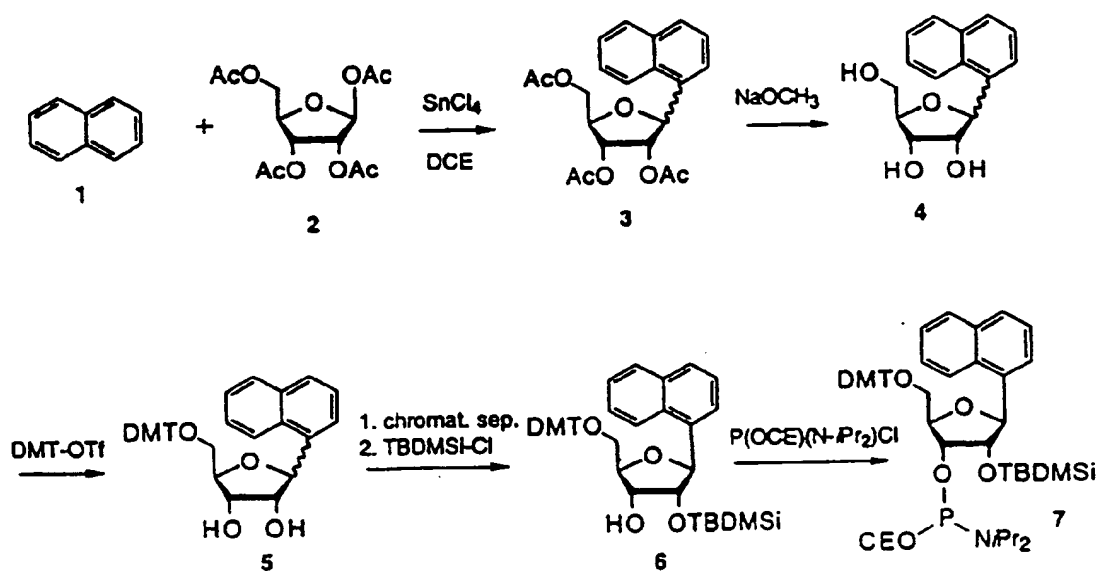
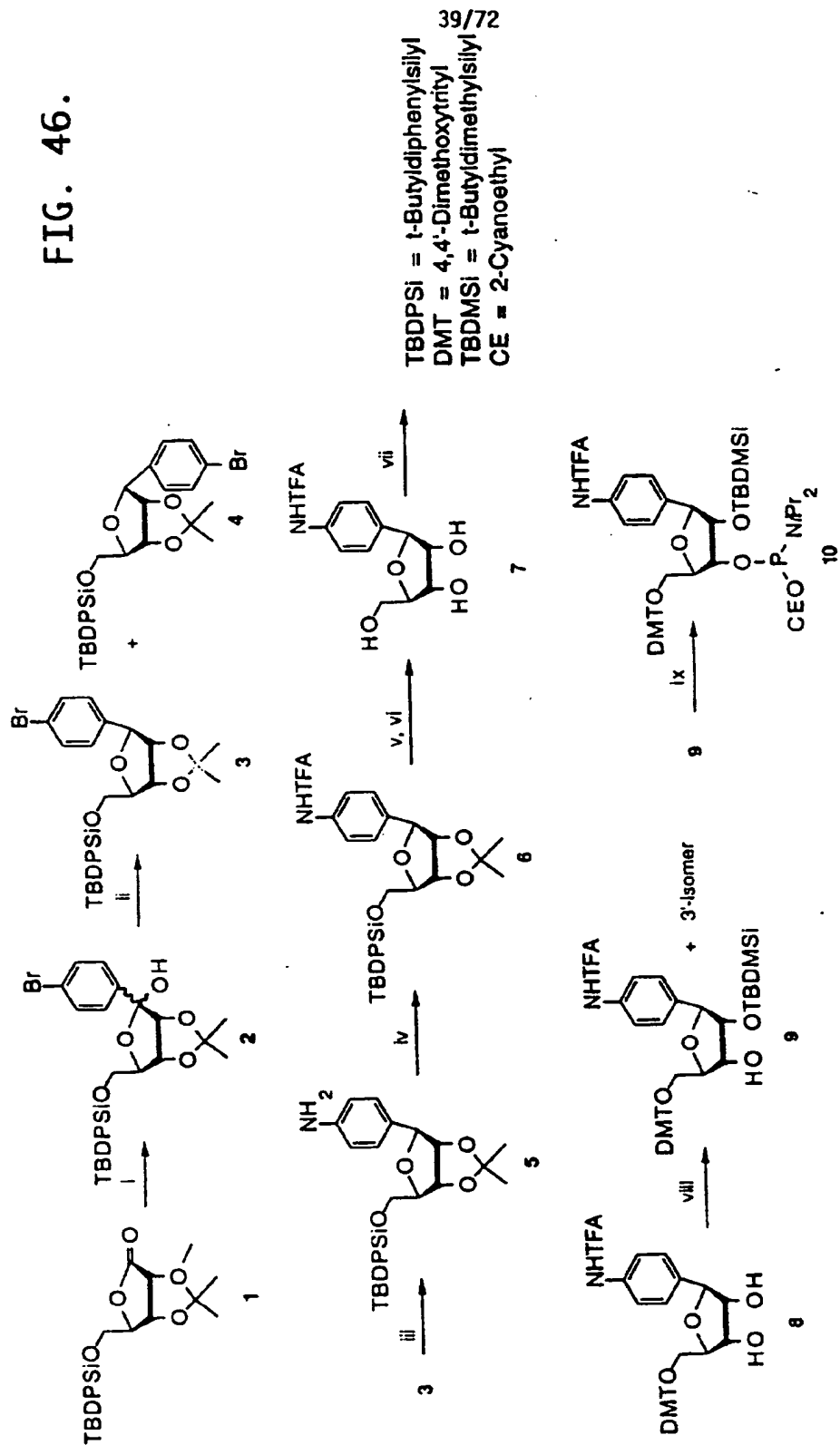


FIG. 45.



SUBSTITUTE SHEET (RULE 26)

FIG. 46.



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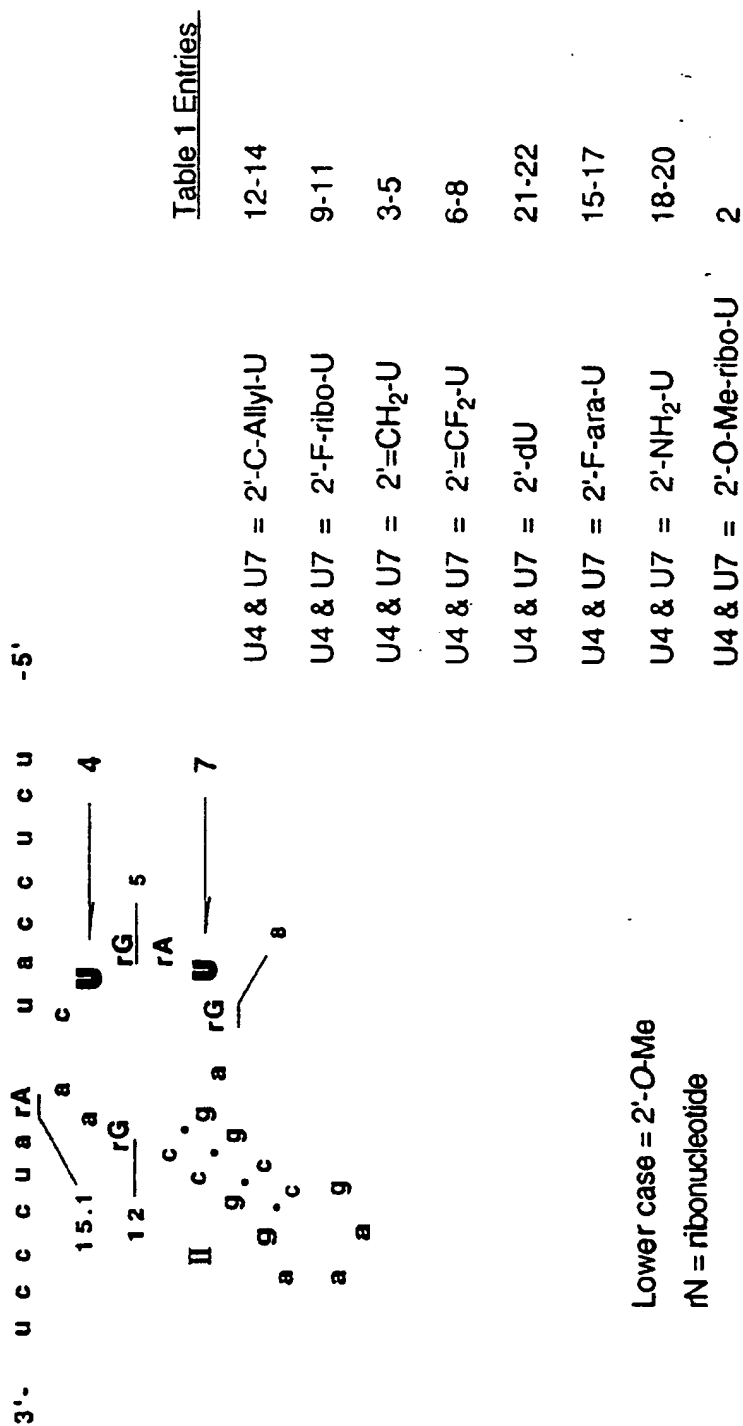
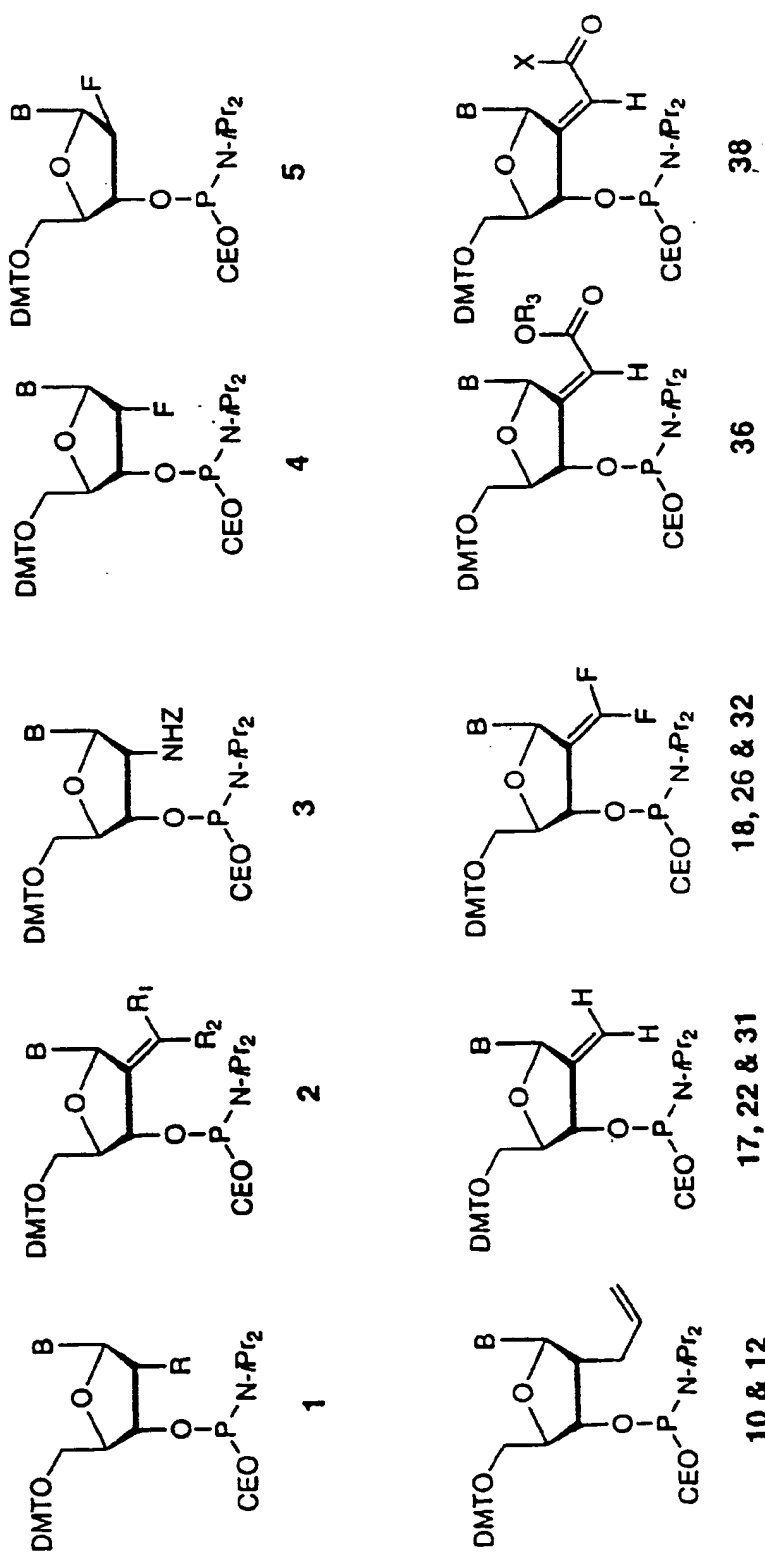


FIG. 47.

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B = Protected A, C, G, U, T, 2AP, I, DiAP, P etc.

FIG. 48.

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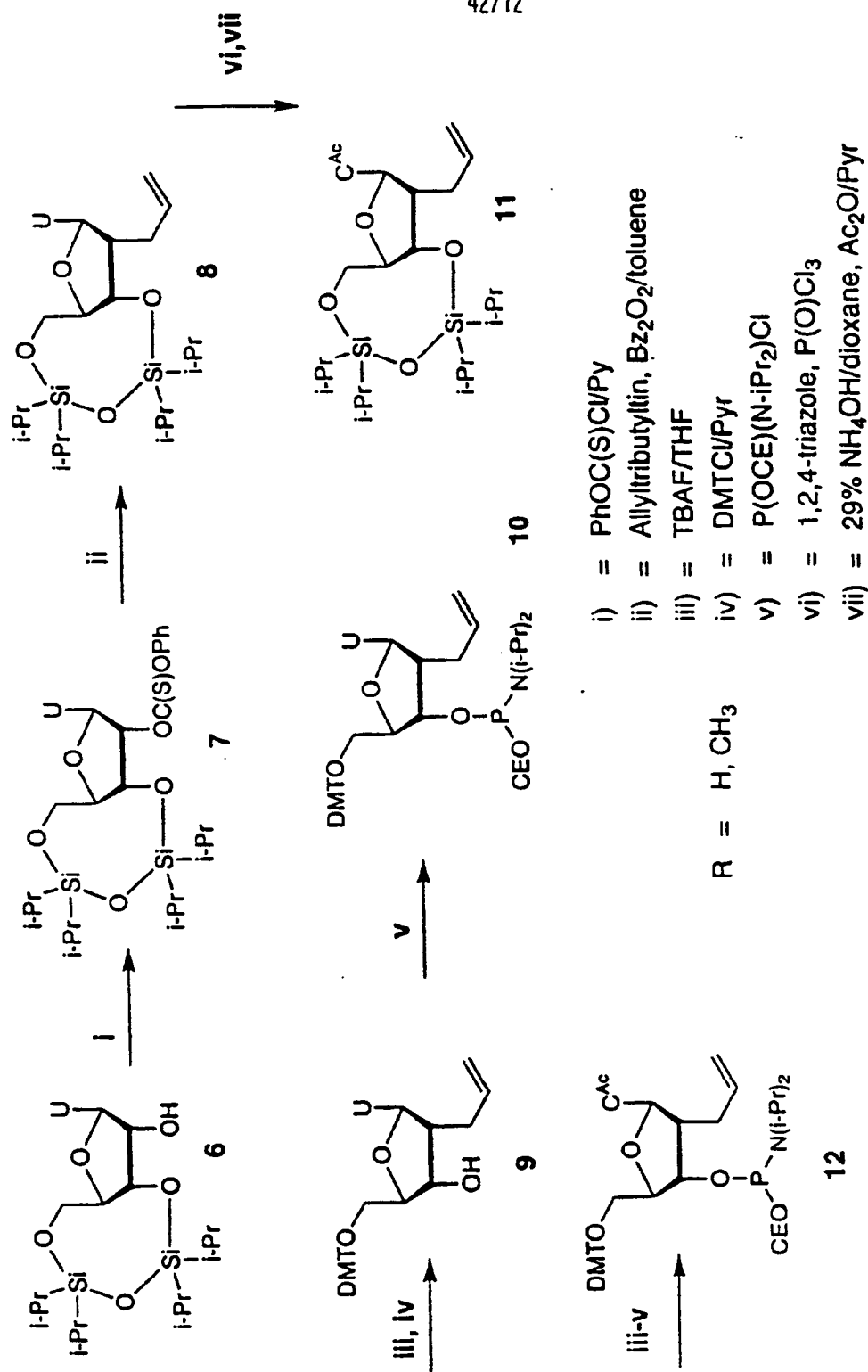
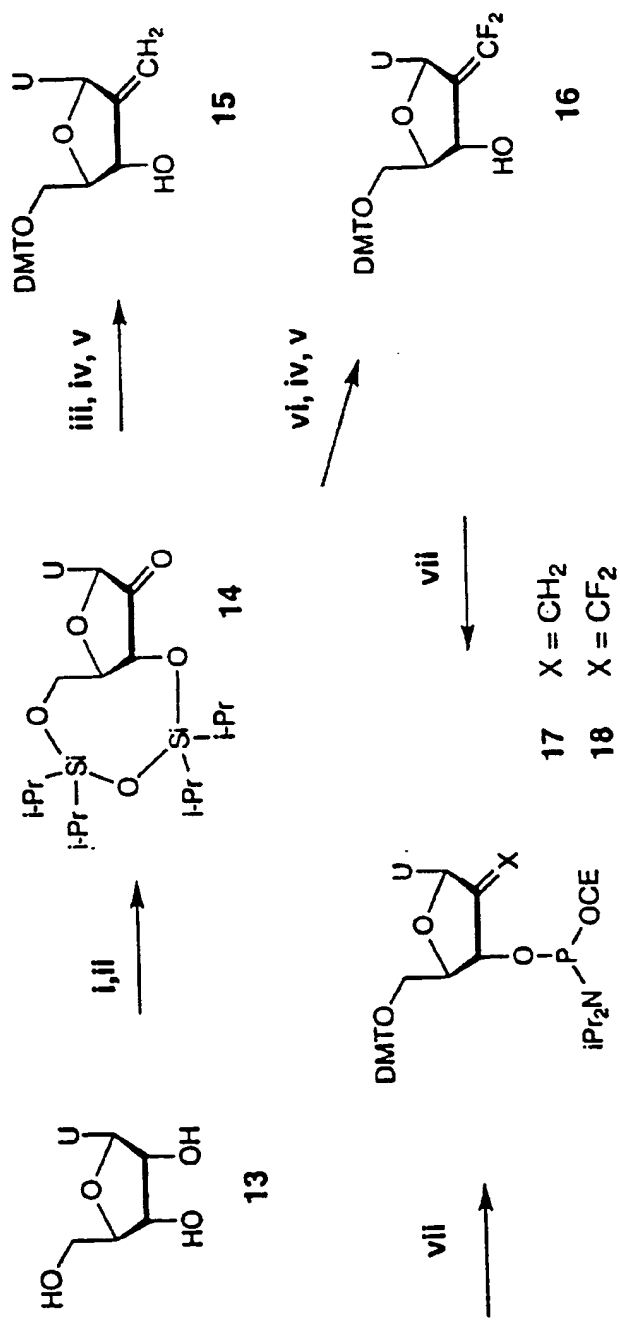


FIG. 49.

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- i) = Markiewicz reagent
 ii) = DMSO & Ac₂O
 iii) = Ph₃PCH₃I
 iv) = TBAF/THF
 v) = DMTCI/Pyr
 vi) = Ph₃P, ClCF₂COONa
 vii) = P(OCE)(N-iPr₂)Cl

FIG. 50.

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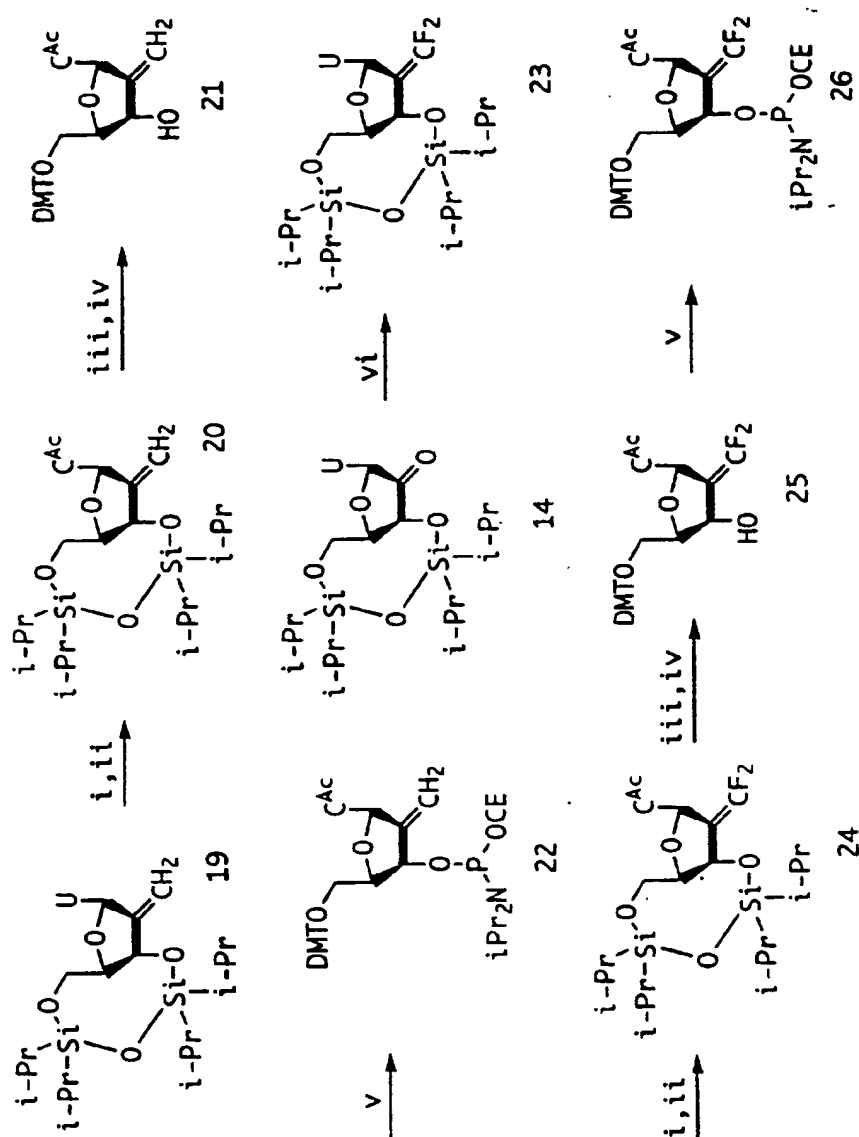


FIG. 51.

SUBSTITUTE SHEET (RULE 26)

i) = 1,2,4-triazole, P(O)Cl₃ iii) = TBAF/THF v) = P(OCE)(N-iPr)₂Cl
 ii) = 29% NH₄OH/dioxane, Ac₂O/Pyr iv) = DMTCl/Pyr vi) = Ph₃P, ClCF₂COONa

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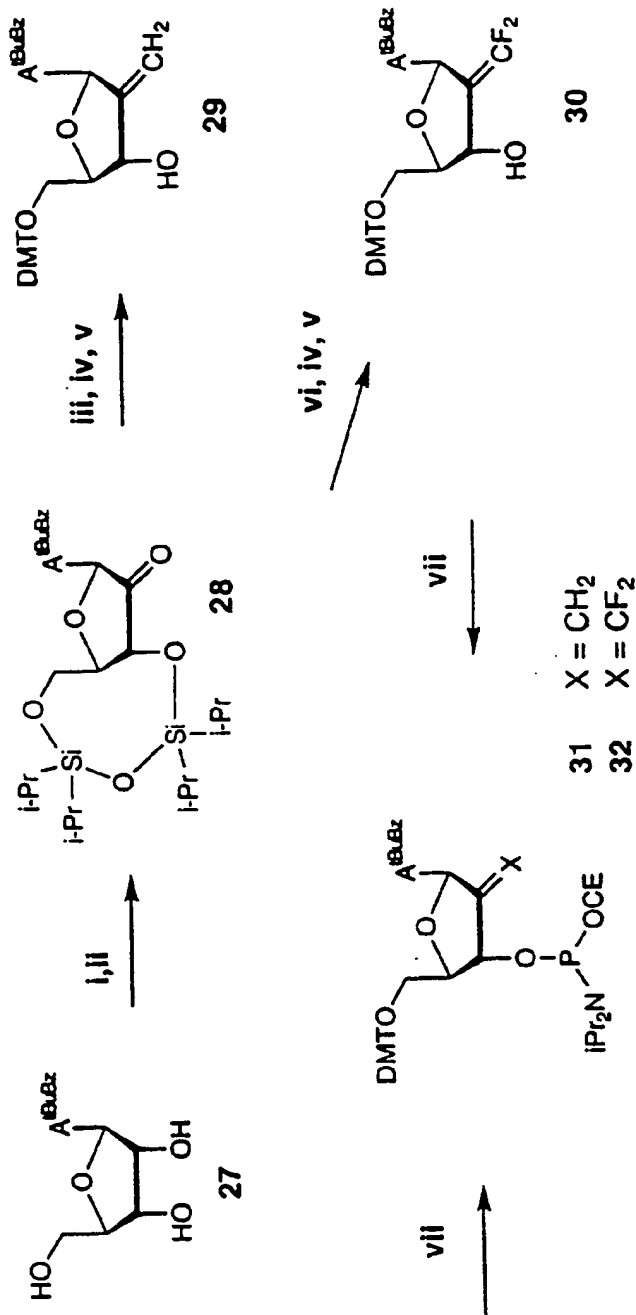
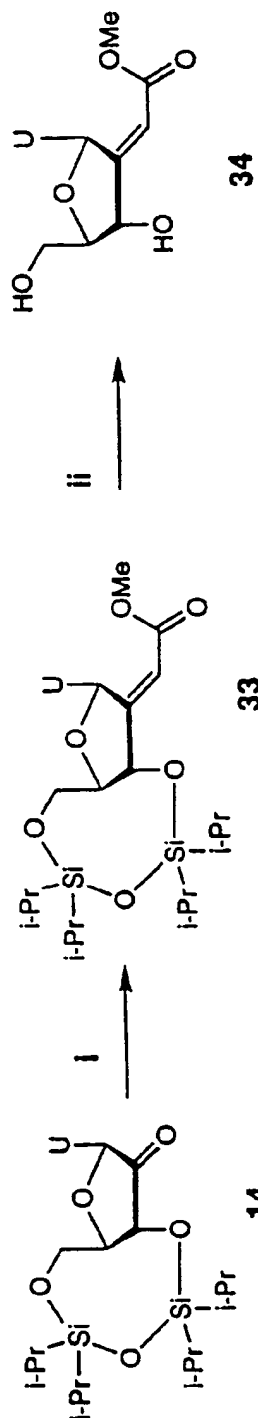


FIG. 52.

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i) = $\text{Ph}_3\text{PC}=\text{CHC}(\text{O})\text{OCH}_3 \cdot \text{OAc}$
 ii) = $\text{NEt}_3 \cdot 3 \text{ HF}$
 iii) = DMTCI/Pyr
 iv) = $\text{P}(\text{OCE})(\text{N-}i\text{Pr}_2)\text{Cl}$
 v) = MeOH/NaOH

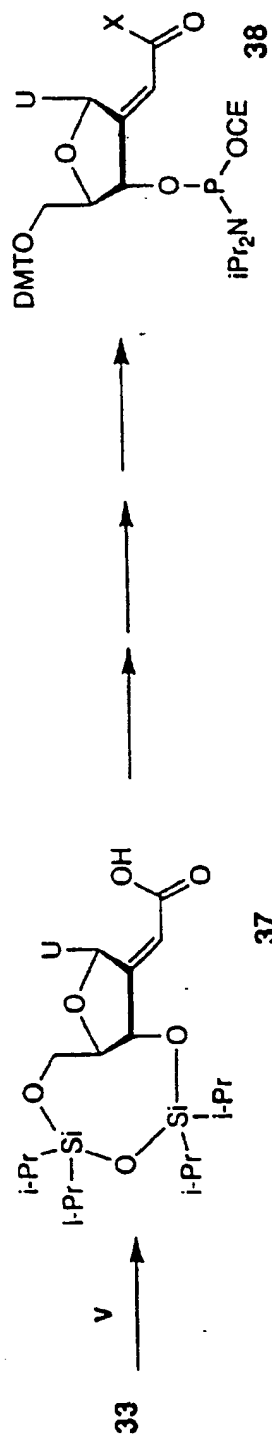
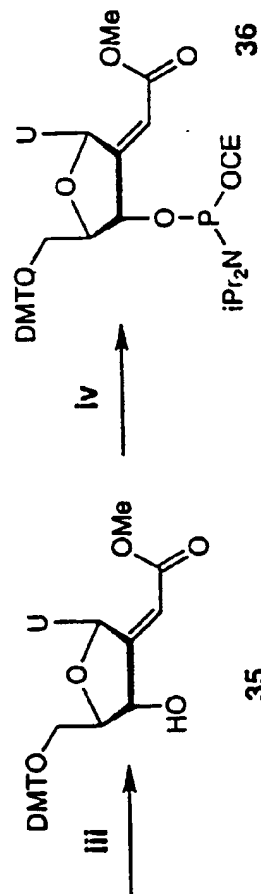
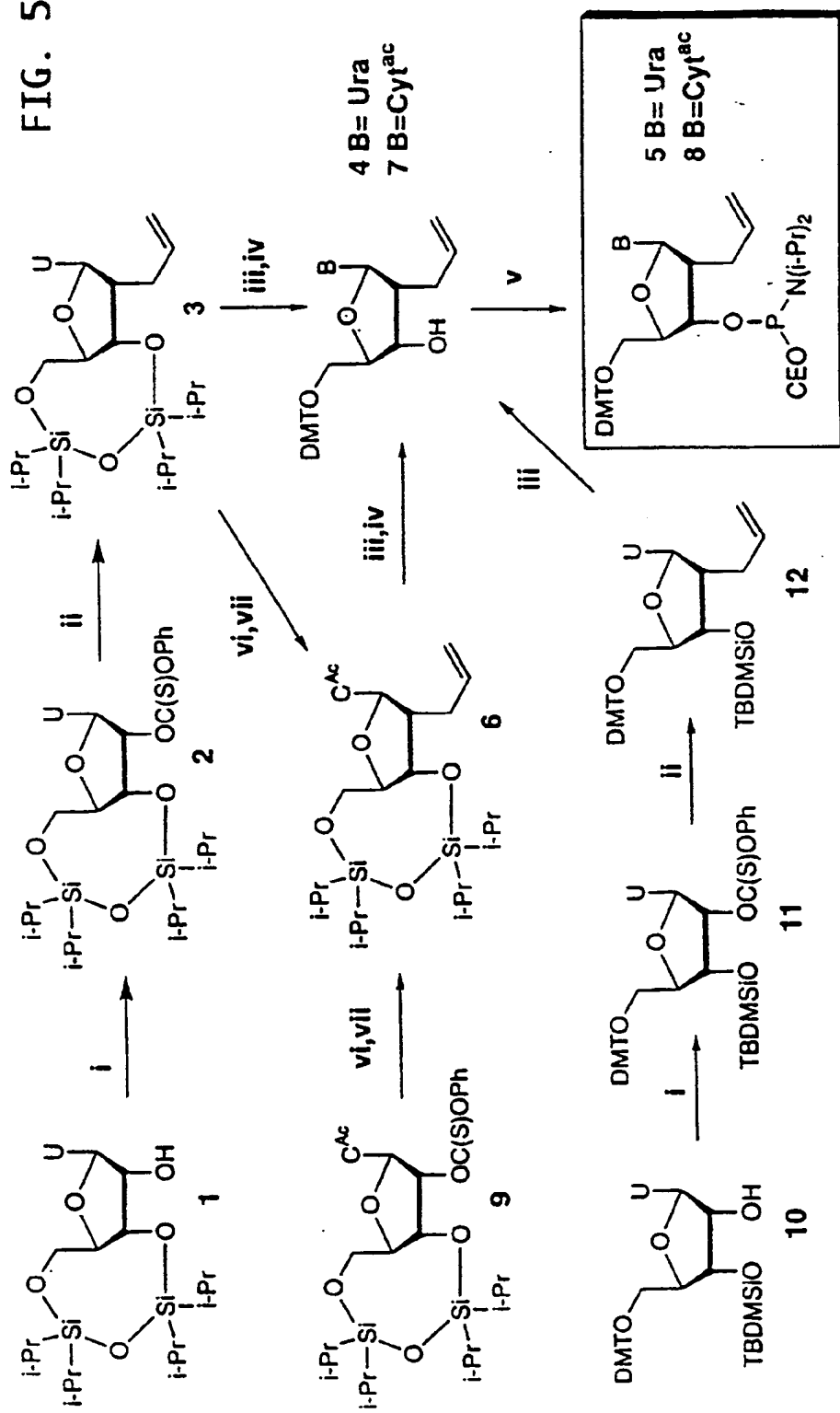


FIG. 53.

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FIG. 54.



SUBSTITUTE SHEET (RULE 26)

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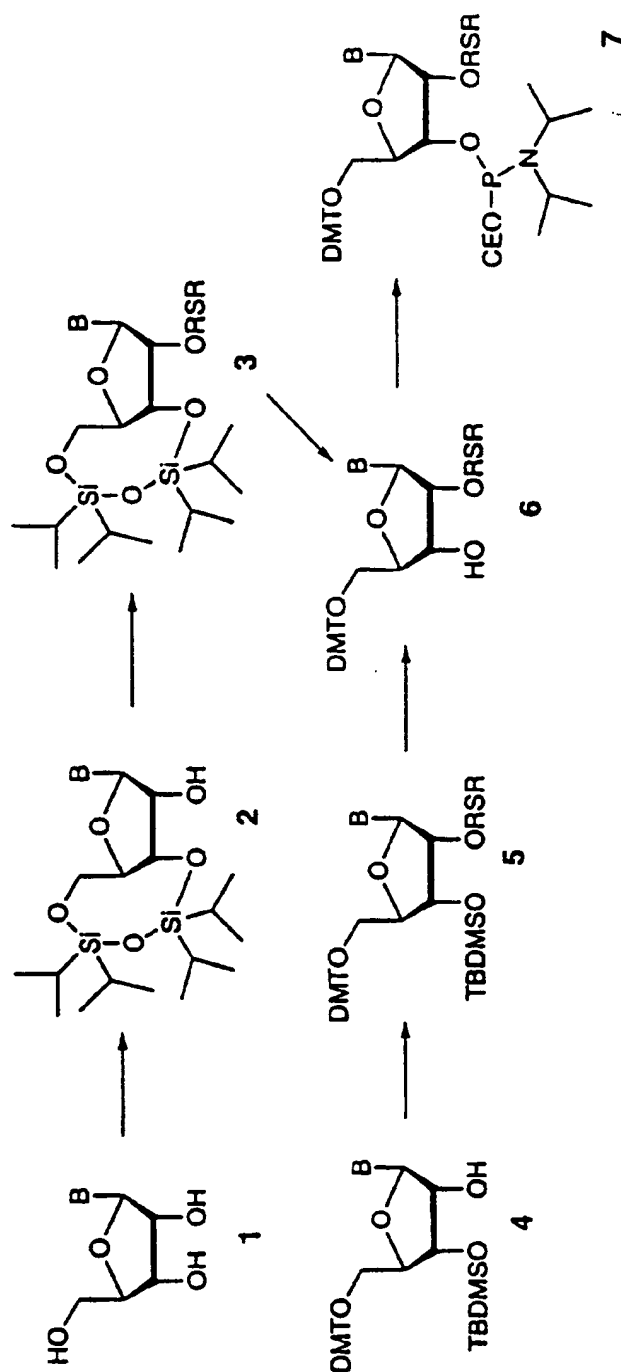


FIG. 55.

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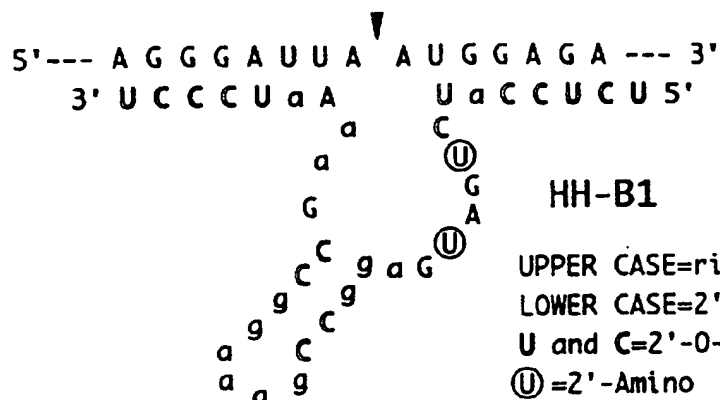


FIG. 56a.

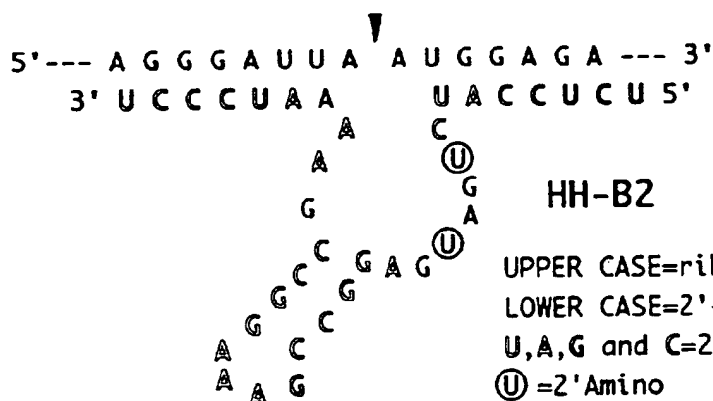


FIG. 56b.

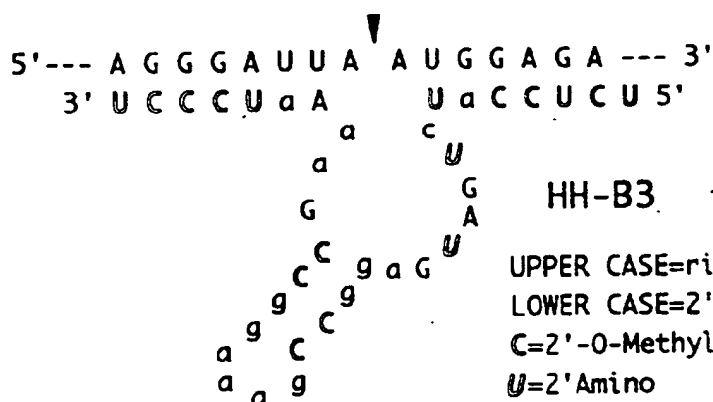


FIG. 56c.

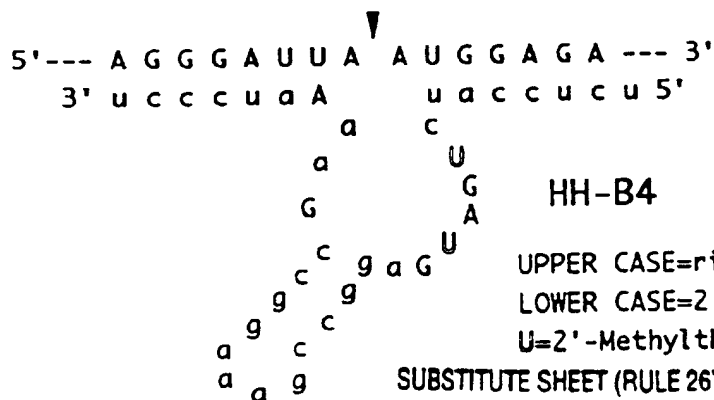


FIG. 56d.

SUBSTITUTE SHEET (RULE 26)

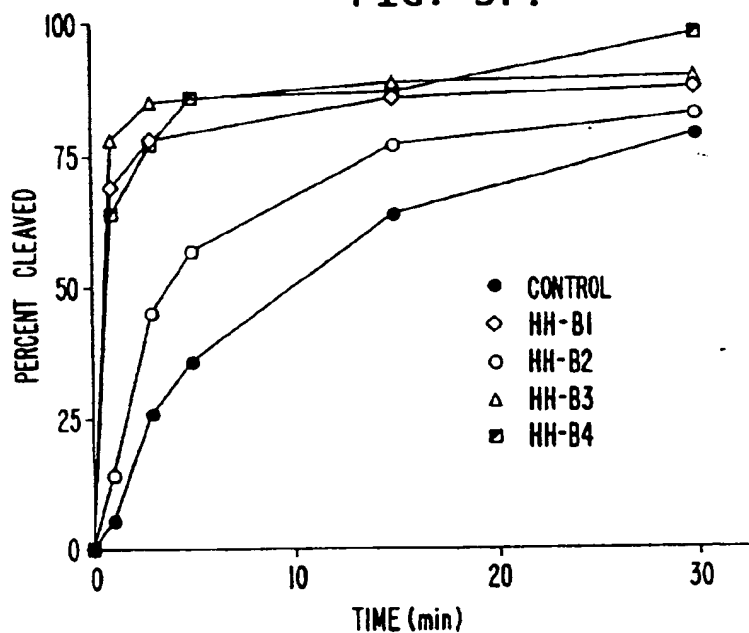
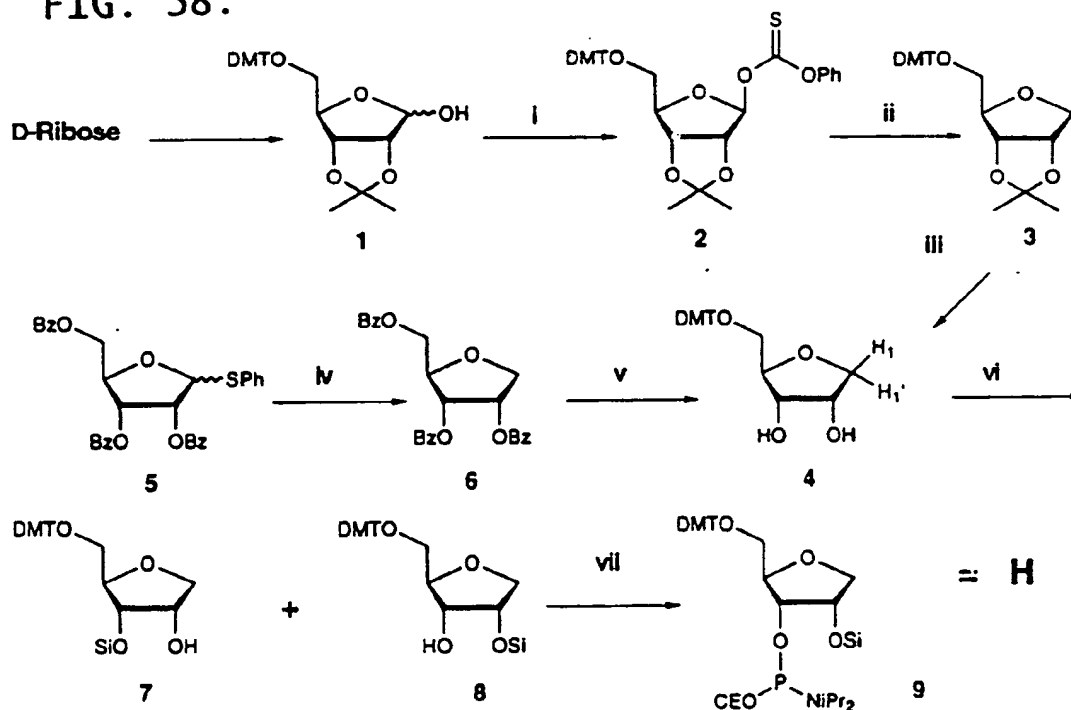
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FIG. 57.

FIG. 58.



Si = *t*-Butyldimethylsilyl
 DMT = 4,4'-Dimethoxytrityl
 CE = Cyanoethyl

Reagents and Conditions: i) PhOC(S)-Cl/DMAP, ii) Bu₃SnH/AIBN, iii) CF₃COOH, DMT-Cl/Pyr, iv) Bu₃SnH/Bz₂O₂, v) 2M NaOH/Pyr/MeOH, DMT-Cl/Pyr, vi) TBDMS-Cl/AgNO₃, vii) P(OCE)(N-*i*Pr₂)Cl
 SUBSTITUTE SHEET (RULE 26)

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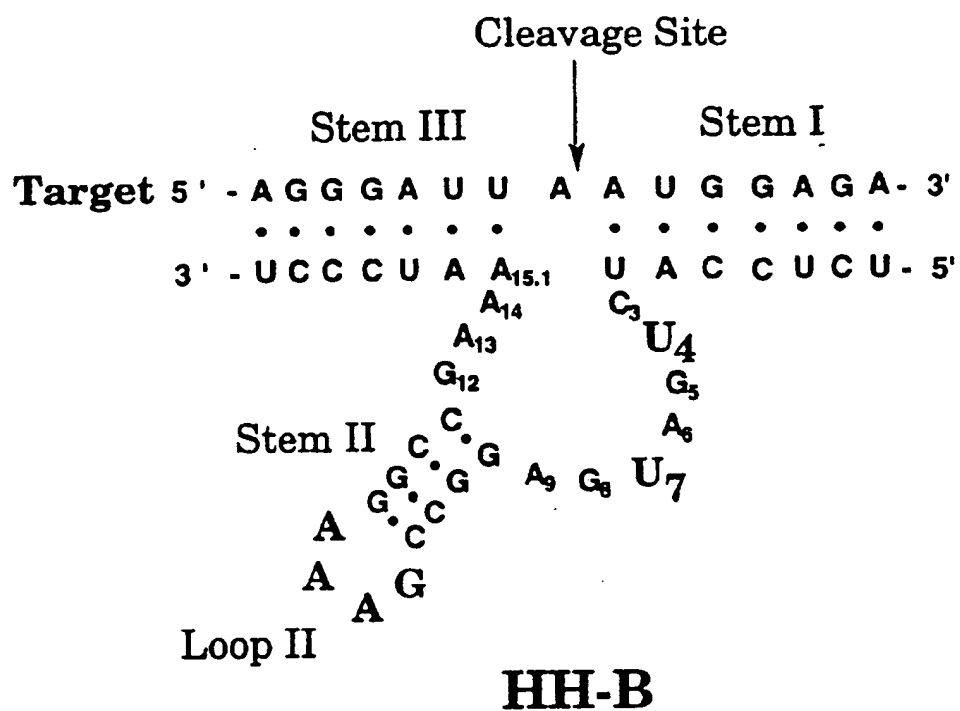


FIG. 59.

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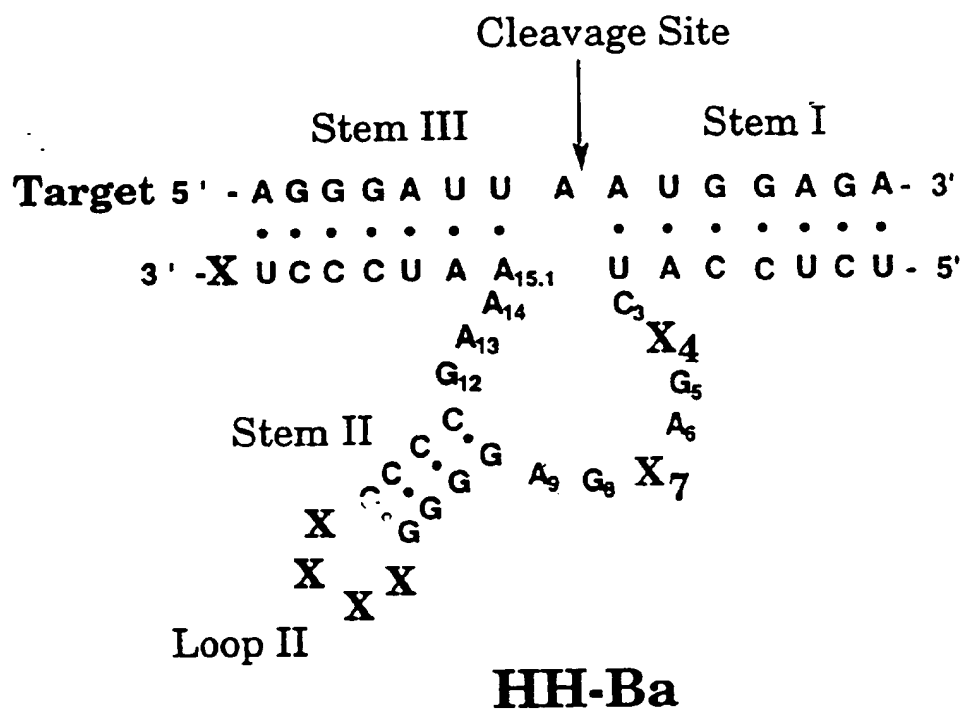


FIG. 60.

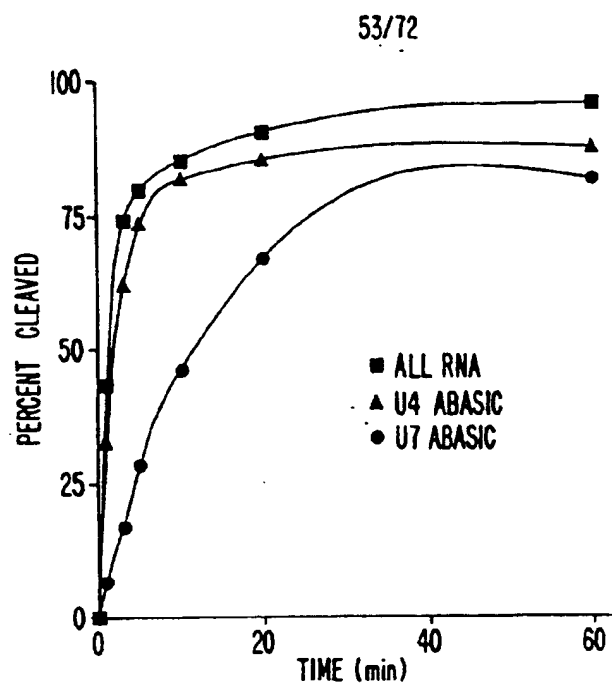


FIG. 61.

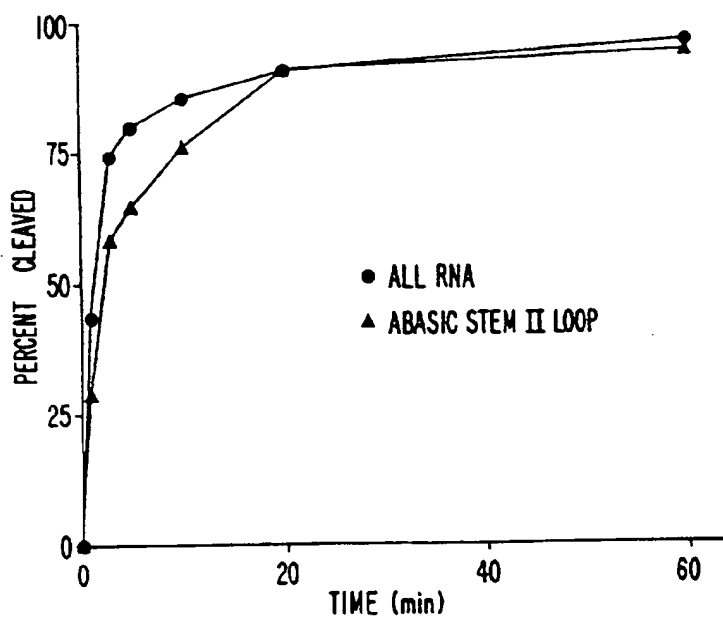


FIG. 62.

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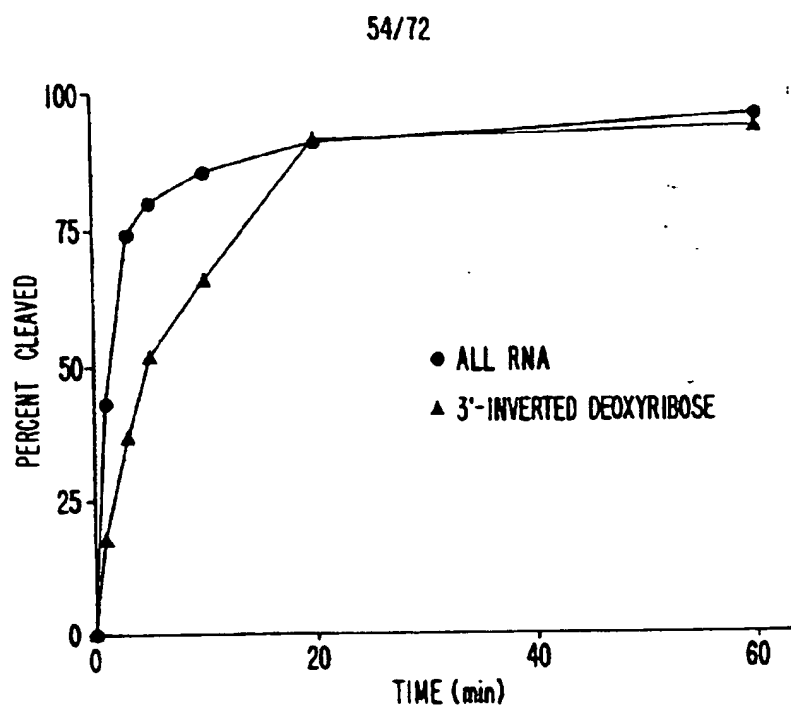


FIG. 63.

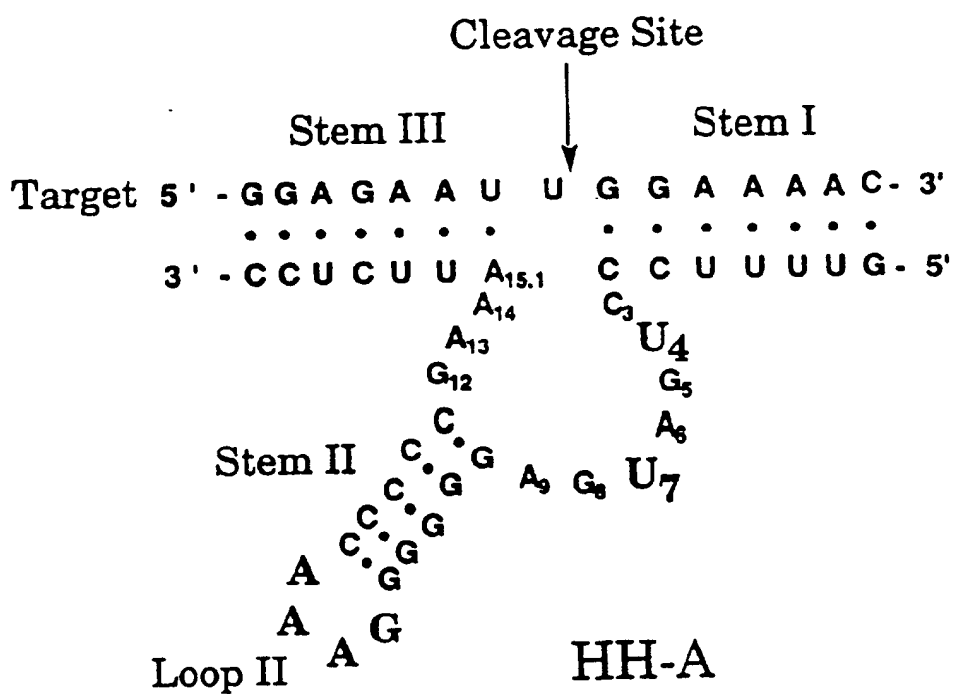


FIG. 64.

SUBSTITUTE SHEET (RULE 26)

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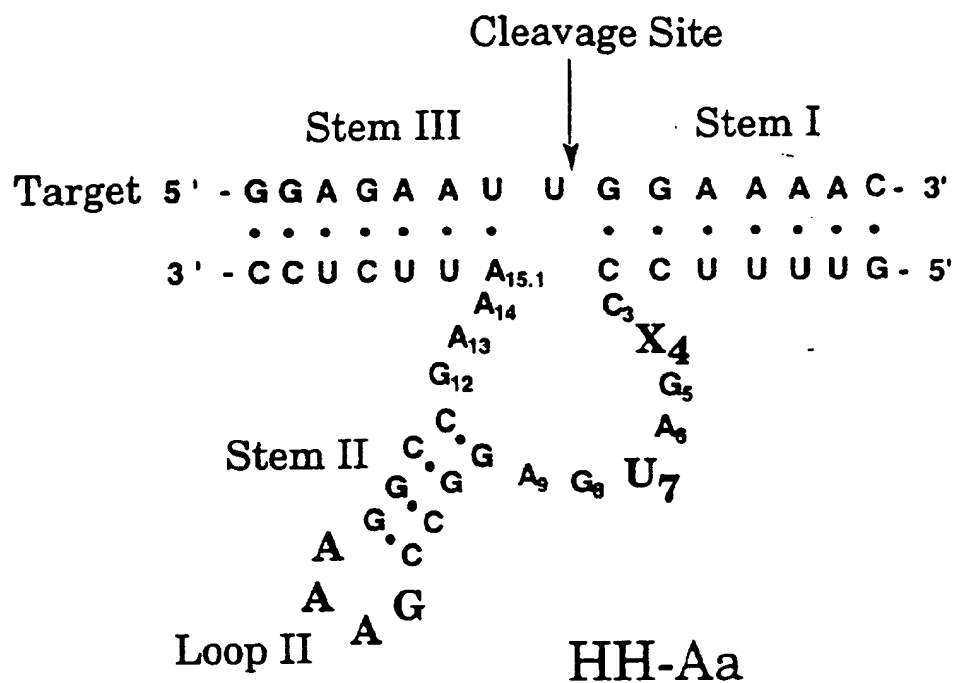


FIG. 65.

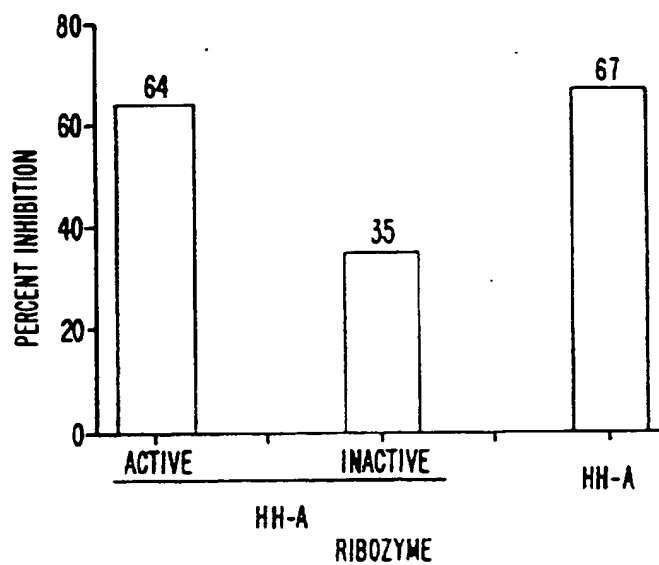


FIG. 66.

SUBSTITUTE SHEET (RULE 26)

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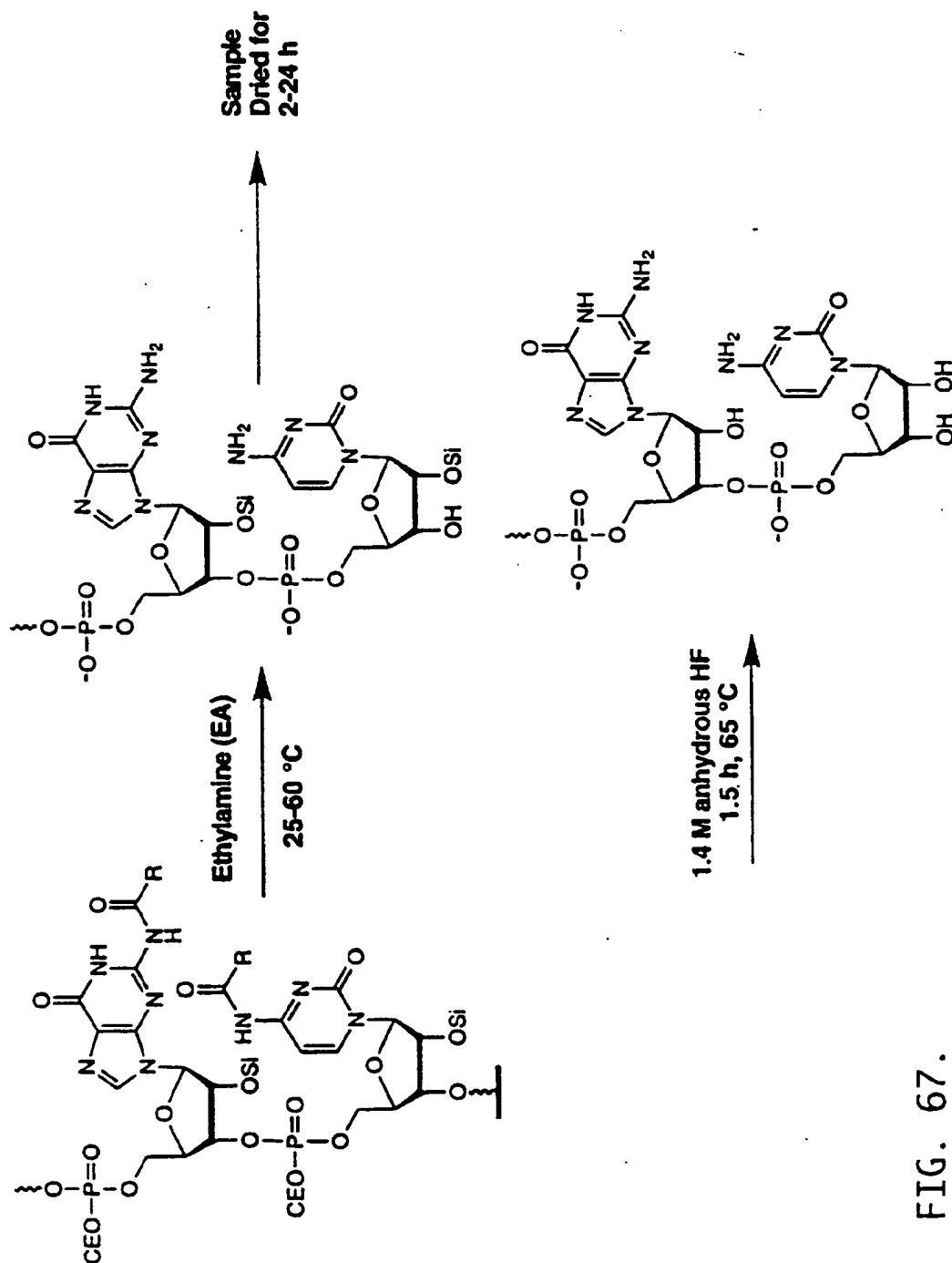


FIG. 67.

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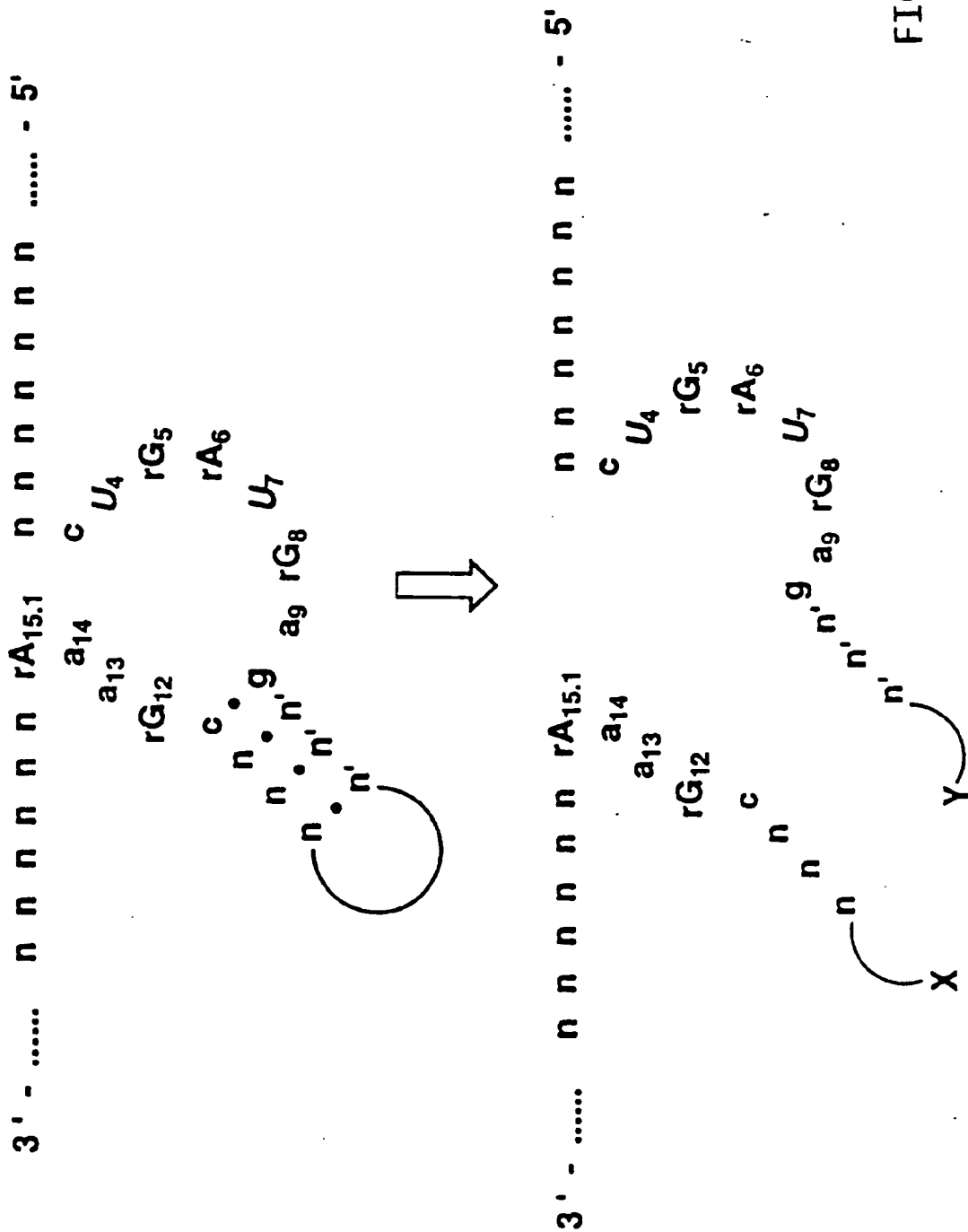
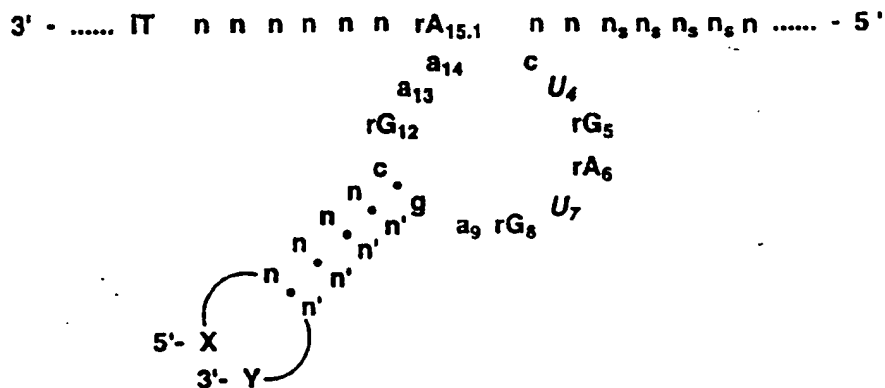


FIG. 68.

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NOTE: $(\text{CH}_2)_n$ refers to any linkage. In addition, X and Y can be interchanged.

$$X = (\text{CH}_2)_n\text{SH}, Y = (\text{CH}_2)_n\text{SH} \rightarrow \text{disulfide}$$
$$X = (\text{CH}_2)_n\text{NHR}, Y = \text{ribose} \longrightarrow \text{morpholino}$$
$$X = (\text{CH}_2)_n\text{NHR}, Y = \text{CO}_2\text{H} \longrightarrow \text{amide}$$
$$X = (\text{CH}_2)_n\text{X}, \quad Y = (\text{CH}_2)_n\text{OH} \longrightarrow \text{ether, X = halogen}$$
$$X = (\text{CH}_2)_n\text{NHR}, Y = \text{CHO} \longrightarrow \text{amine}$$
$$X = (\text{CH}_2)_n\text{PPh}_3, Y = \text{CHO} \longrightarrow \text{double bond}$$

X = (CH₂)_nNHR, Y = (CH₂)_nSO₂Cl → sulfonamide

$$X = (\text{CH}_2)_n\text{OH}, Y = \text{CO}_2\text{H} \longrightarrow \text{ester}$$
$$X = (\text{CH}_2)_n\text{X}, \quad Y = (\text{CH}_2)_n\text{SH} \longrightarrow \text{thioether, } X = \text{halogen}$$
$$X = (\text{CH}_2)_n\text{COX}, \quad Y = (\text{CH}_2)_n\text{OH} \rightarrow \text{carbonate, } X = \text{halogen}$$

FIG. 69.

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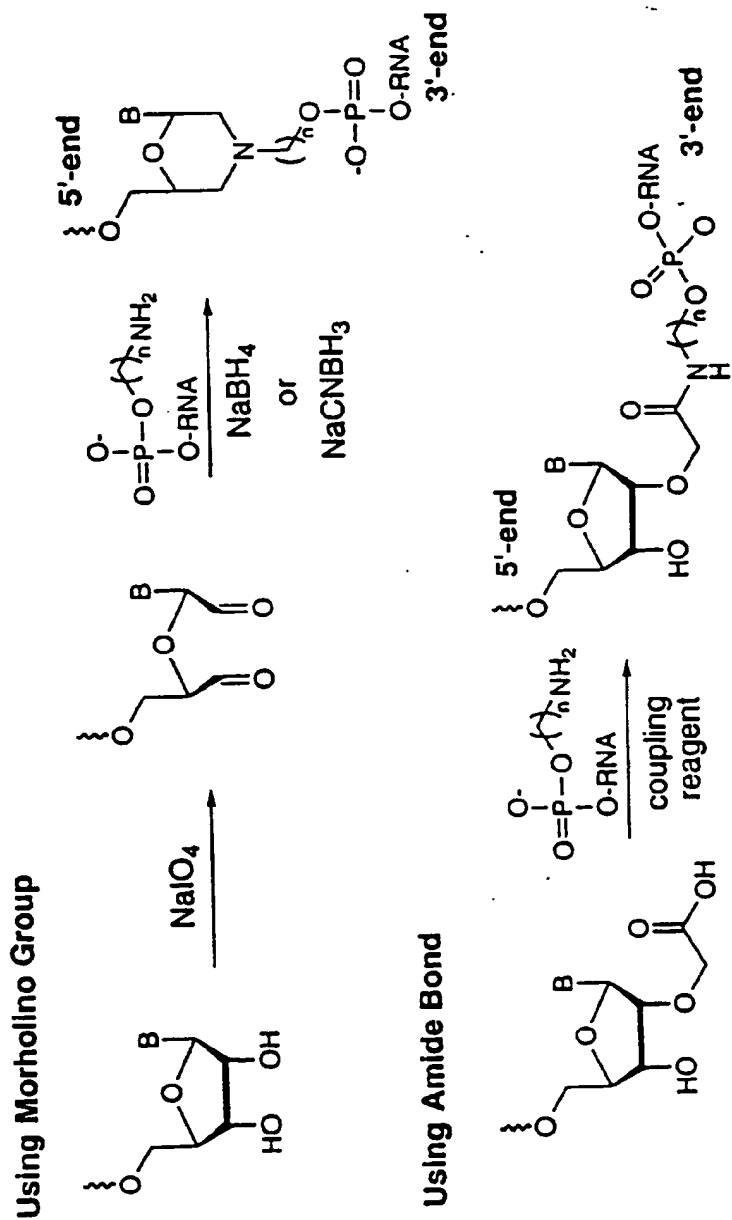


FIG. 70.

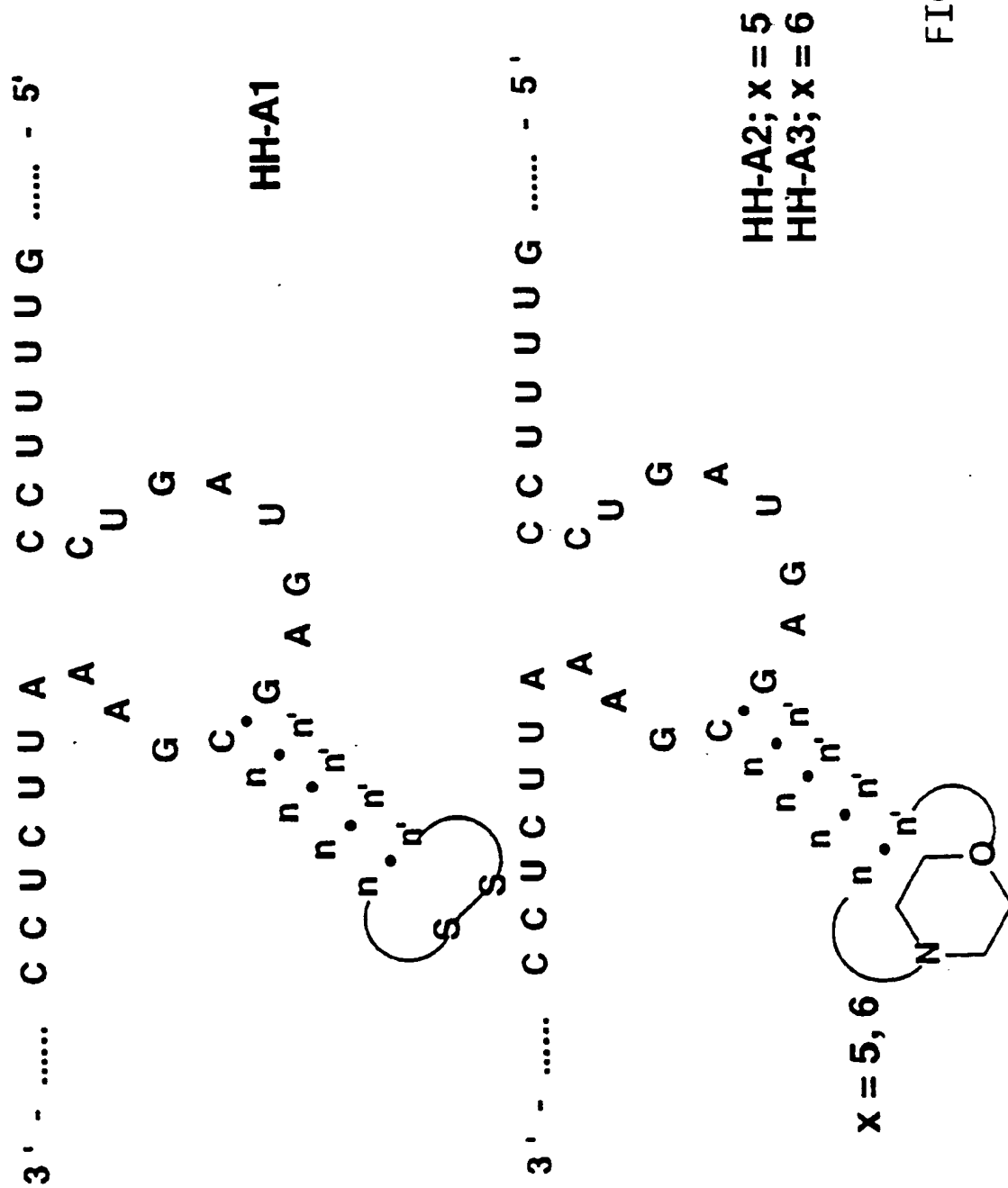


FIG. 71.

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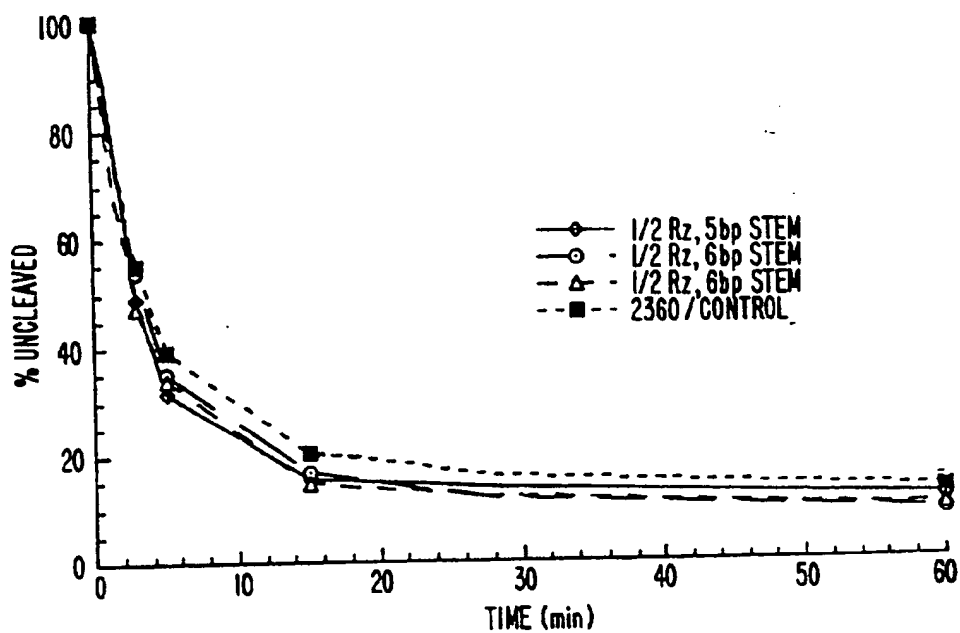


FIG. 72.

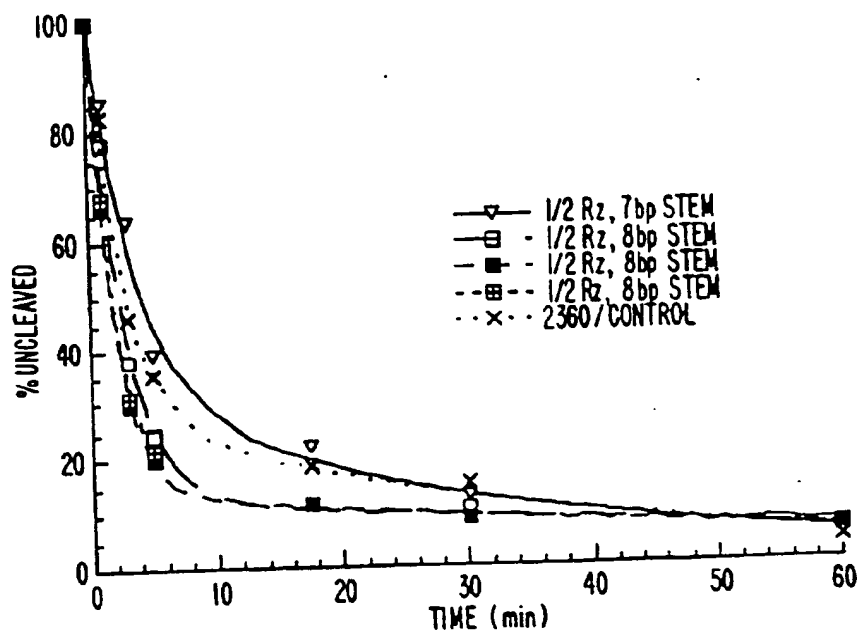


FIG. 73.

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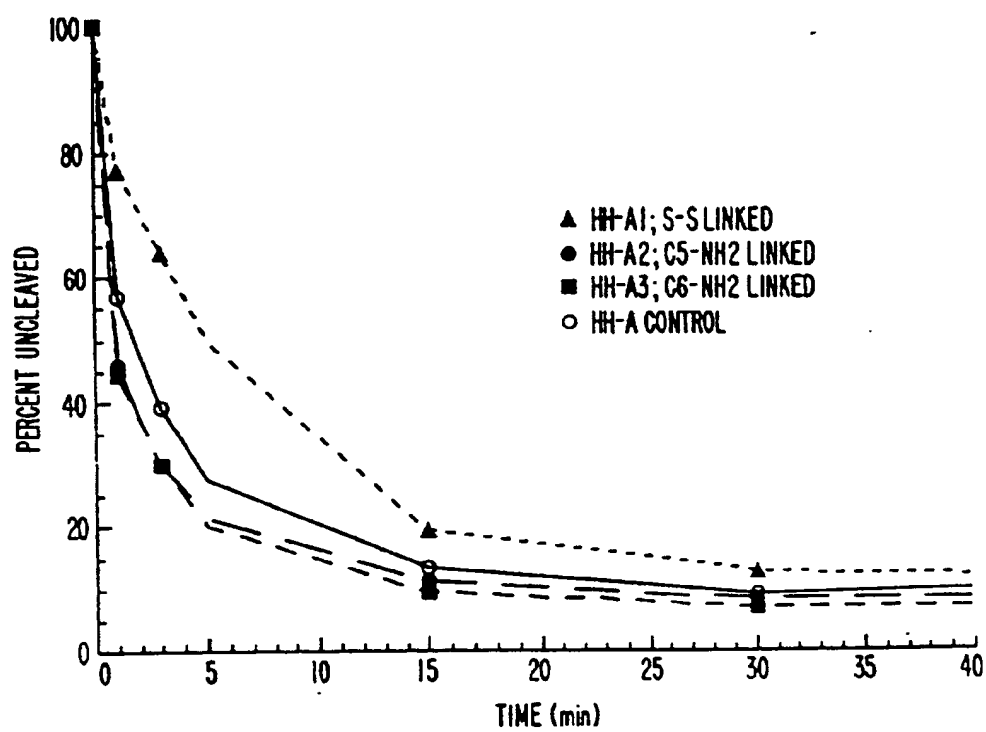
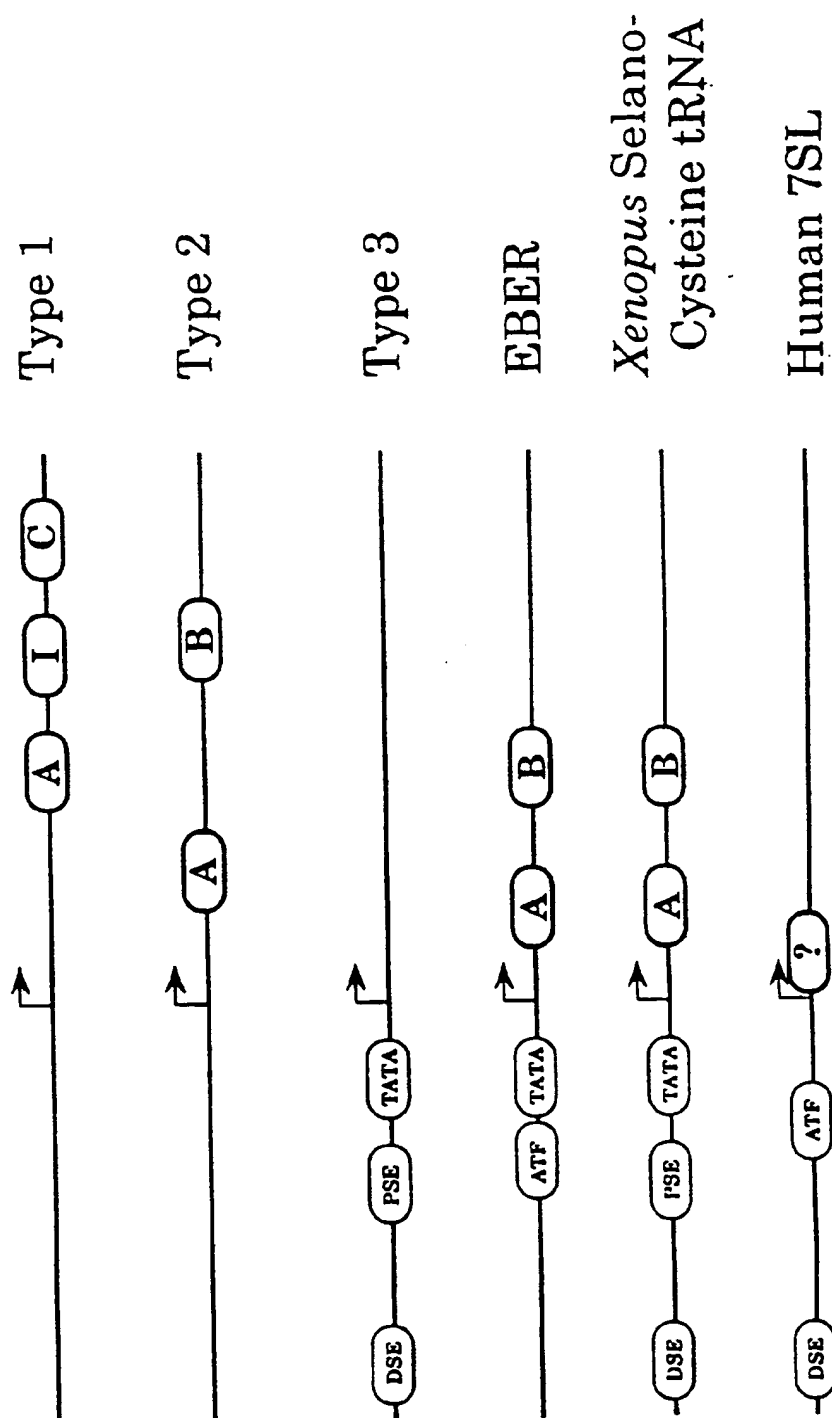


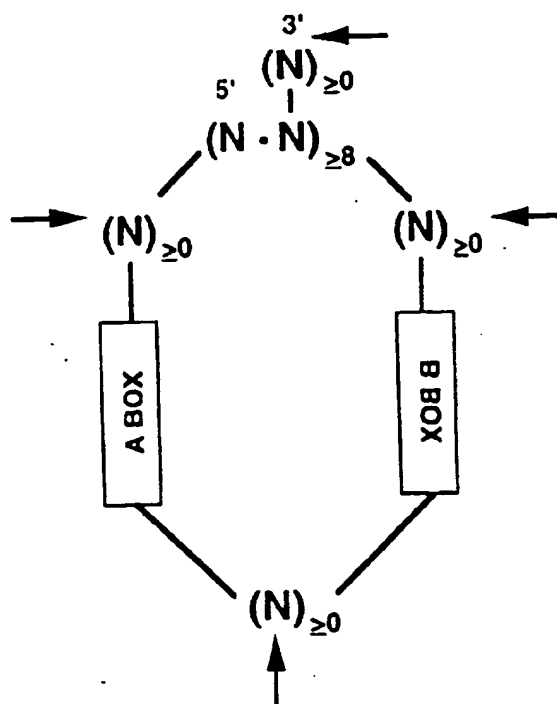
FIG. 74.

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FIG. 75.



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A BOX = URGCNAGYGG

B BOX = GGUUCGANUCC

This is based on Geiduschek & Tocchini-Valentini, (1988) Annu. Review Biochem. 57, 873-914. However this consensus sequence is not meant to be limiting

N = A, U, G, or C

R = Purine

Y = Pyrimidine

• = Indicates base-pairing

— = Indicates covalent linkage

➔ = Indicates sites at which desired RNAs can be cloned

FIG. 76.

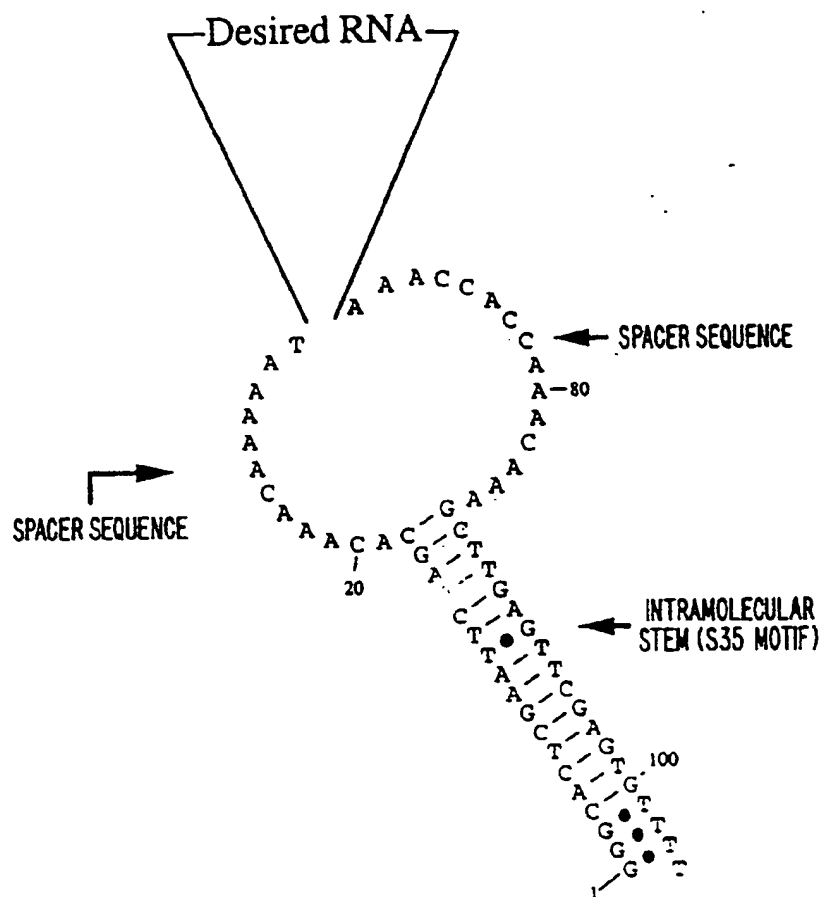
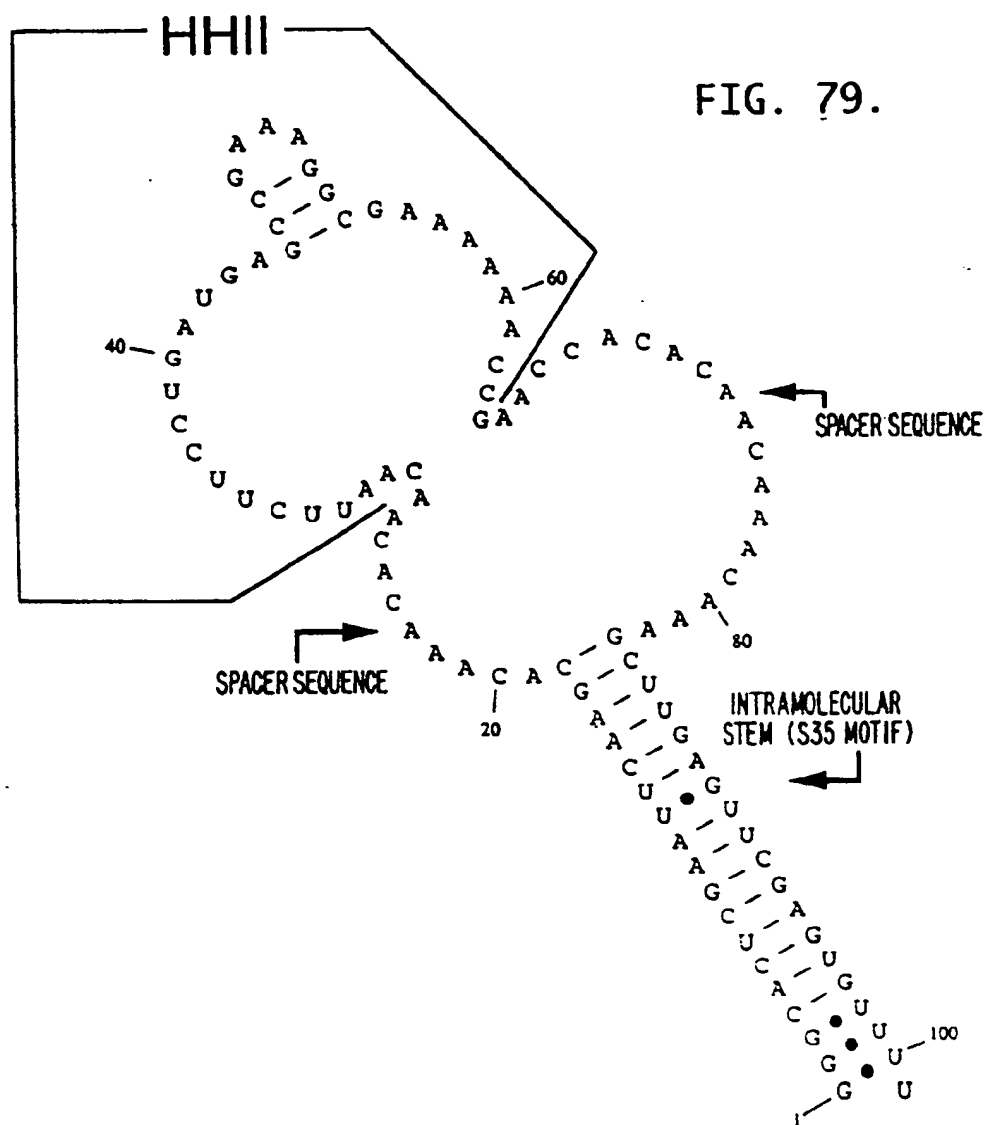


FIG. 77.

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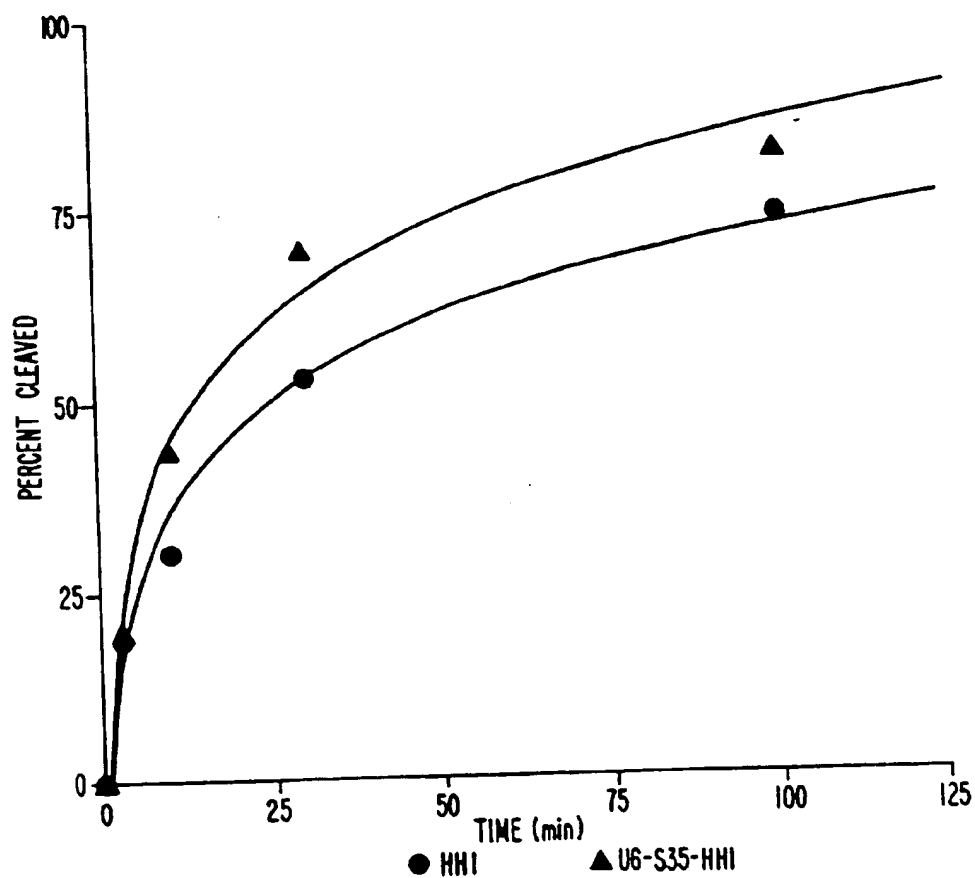


FIG. 80.

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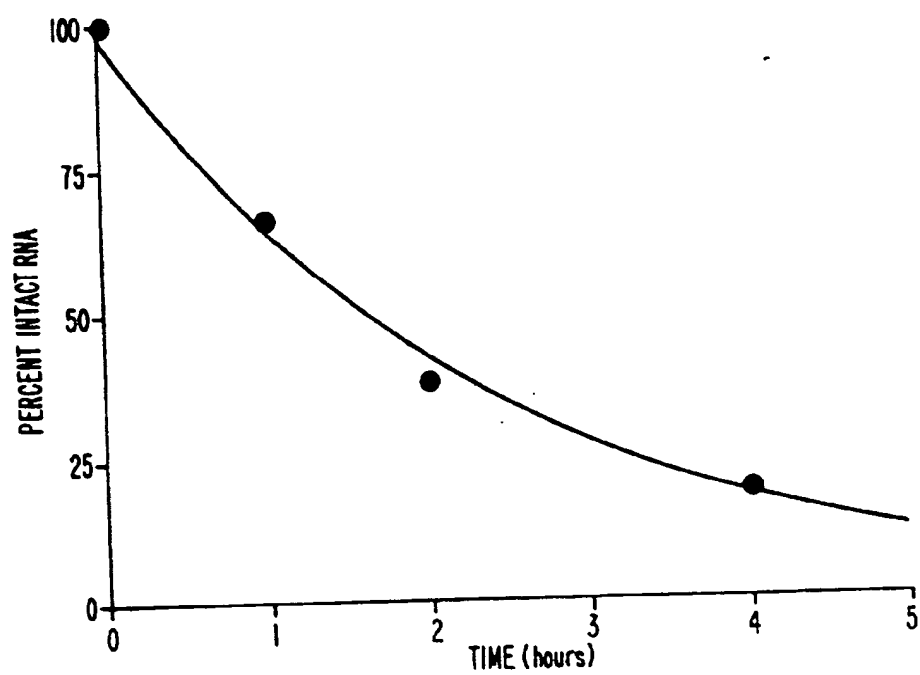


FIG. 81.

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FIG. 82.

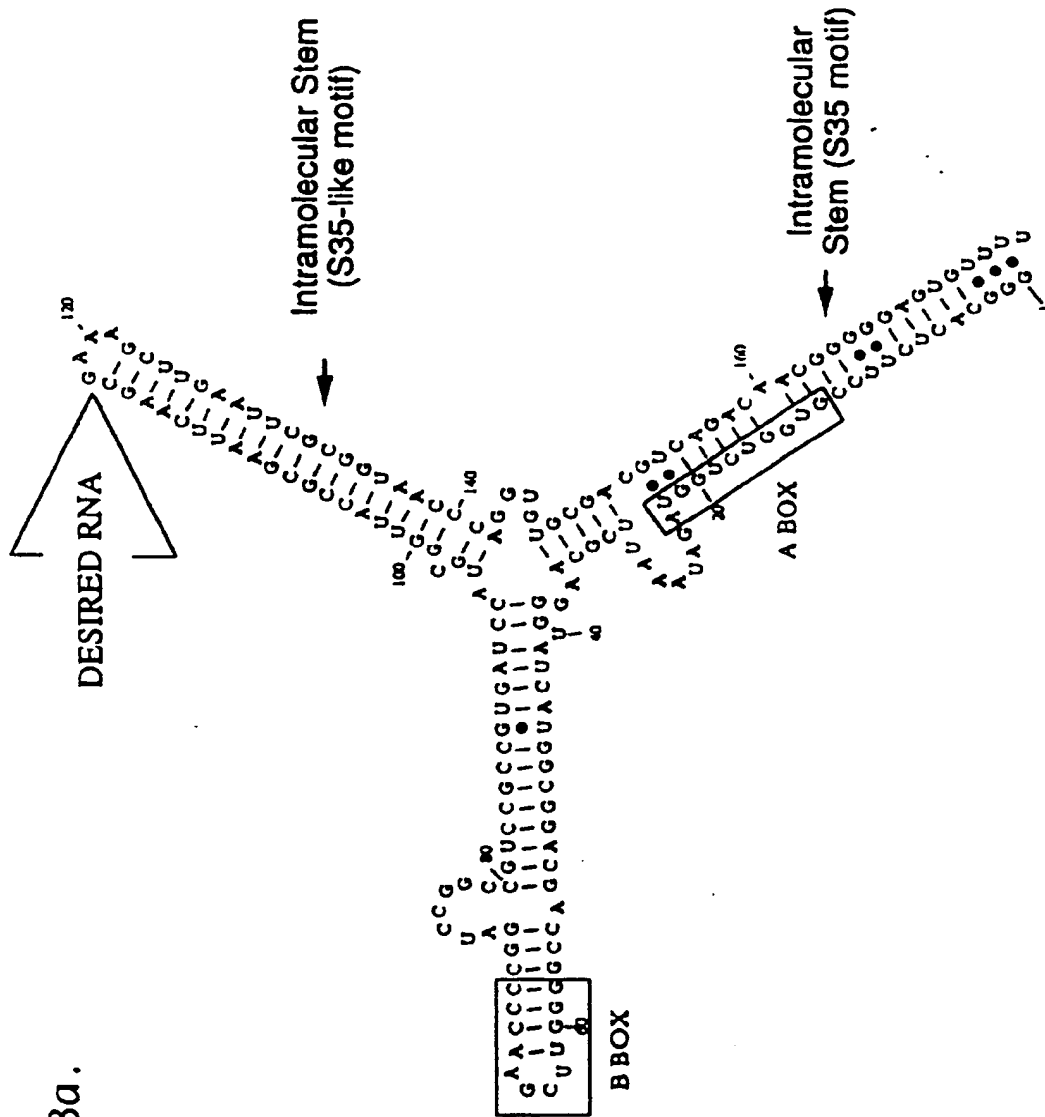
Apical Stem-loop

Central Domain

Terminal Stem

SP G-CCUUUⁿ
SUBSTITUTE SHEET (RULE 26)

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VA1-Chimera

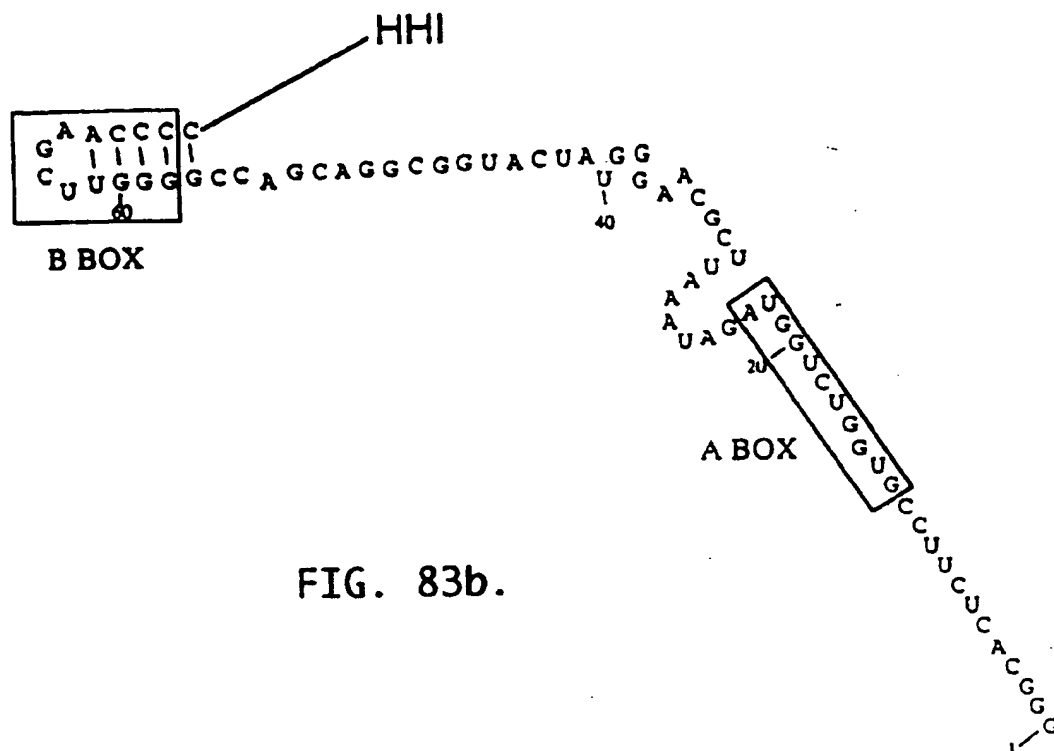
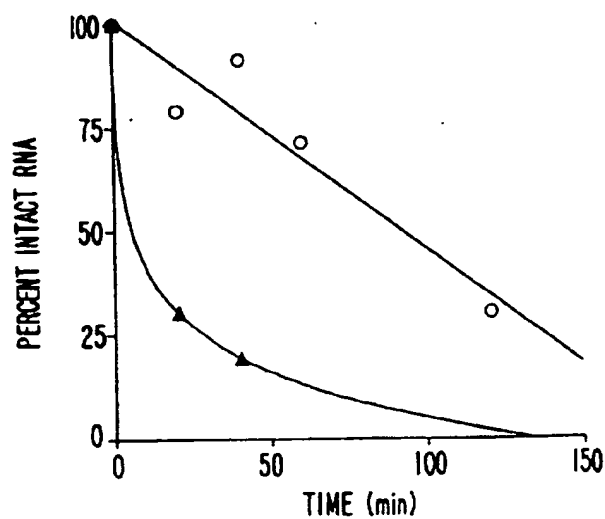


FIG. 83b.



▲ VAI-CHIMERA

○ VAI-S35-CHIMERA

FIG. 84.

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